TheScientist
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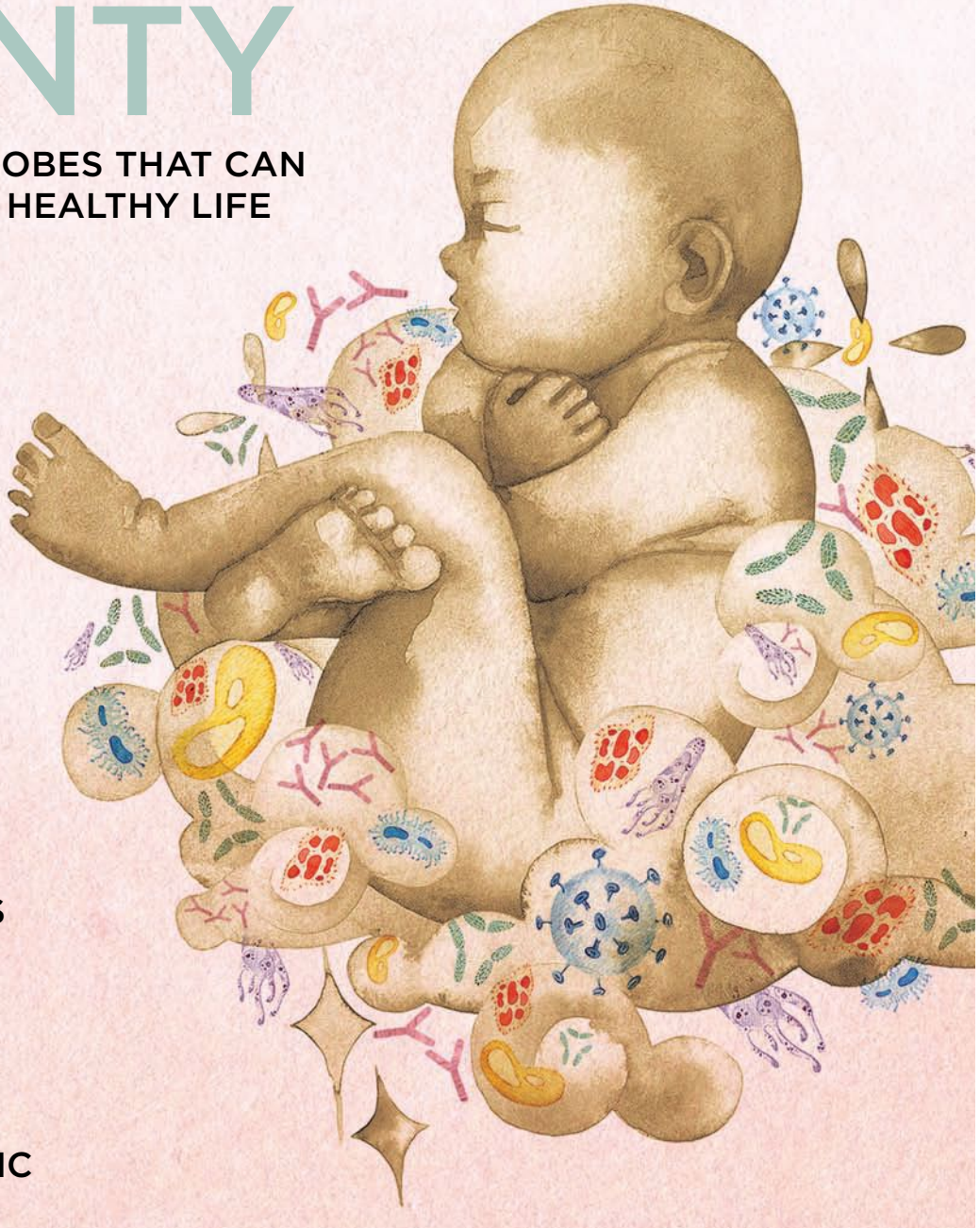
EXPLORING LIFE, INSPIRING INNOVATION

# AN INFANT'S BOUNTY

## BABIES AMASS MICROBES THAT CAN PAVE THE WAY TO A HEALTHY LIFE



LEFT-HANDED DNA AS DYNAMIC CODE

SIDE GIGS FOR ANCIENT ENZYMES

WHEN YOUR SUPERVISOR CHEATS

PLUS
THE 1957 INFLUENZA PANDEMIC

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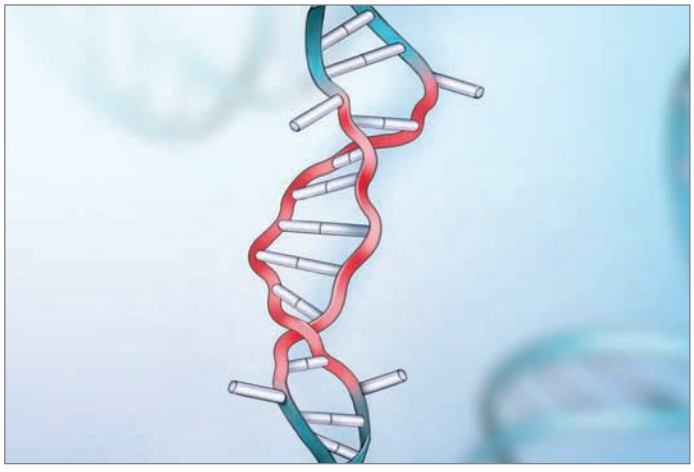

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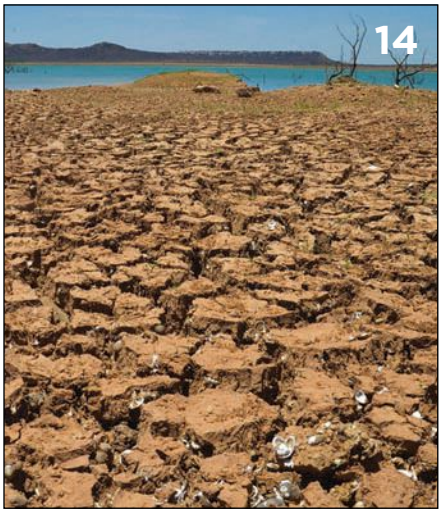


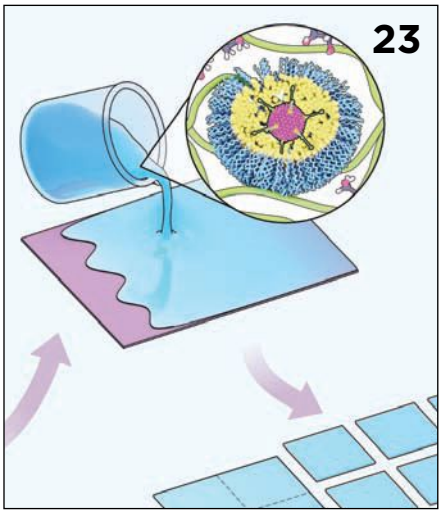





**CORRECTION:**
In the May profile "Unravelling Memory's Mysteries," Elizabeth Buffalo began looking for jobs five years into her postdoc, not eight years, as originally reported. *The Scientist* regrets the error.

PUZZLE ON PAGE 12

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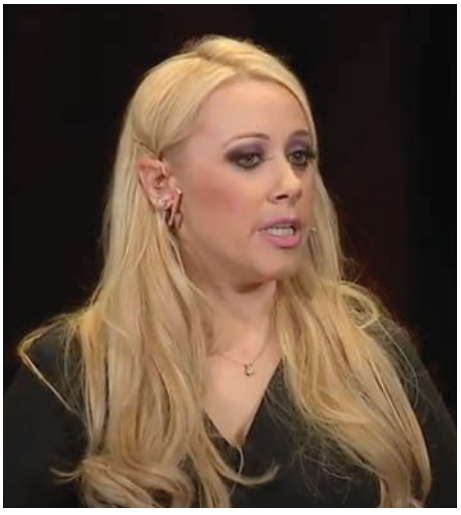

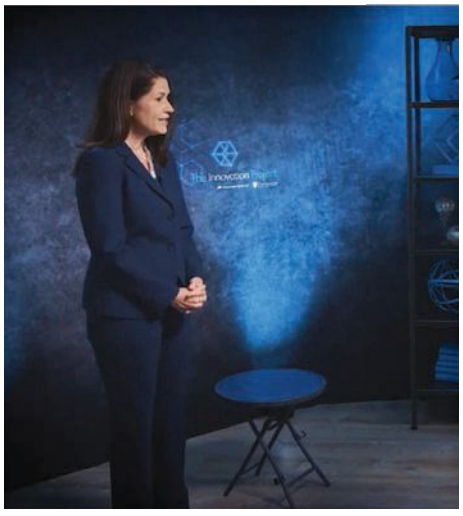

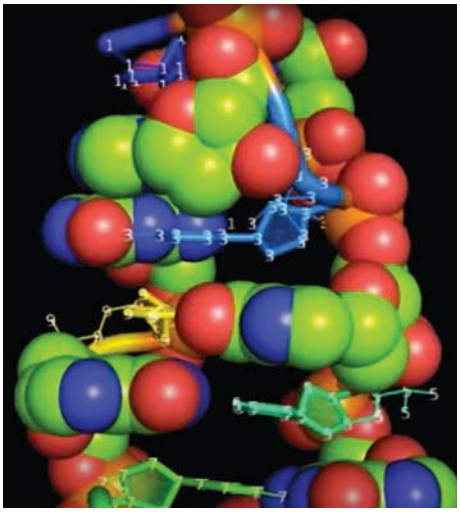

THIS MONTH AT THE-SCIENTIST.COM:

VIDEO
**Early Warning**
Watch this month's Scientist to Watch, Janelle Ayers, discuss the microbiome's role in battling infectious disease.

VIDEO
**Mother Knows Best**
Neonatologist and epidemiologist Juliette Madan discusses her work investigating the effects of the infant microbiome on babies' health outcomes.

VIDEO
**The Other DNA**
See a 3-D model of Z-DNA, the subject of a feature article in this month's issue.

AS ALWAYS, FIND BREAKING NEWS EVERY DAY ON OUR WEBSITE.

# Coming in the July/August issue

- Social isolation has many effects on cognitive health. Researchers are just beginning to piece together the mechanisms.
- Predicting the future of the ongoing pandemic depends on R0, the mathematical term that estimates how infectious the virus is. But R0 has its limitations.
- The pharma industry pivots to respond to COVID-19.
- $CD8^+$ T cells in tumors could be key to making immunotherapies work for more patients.

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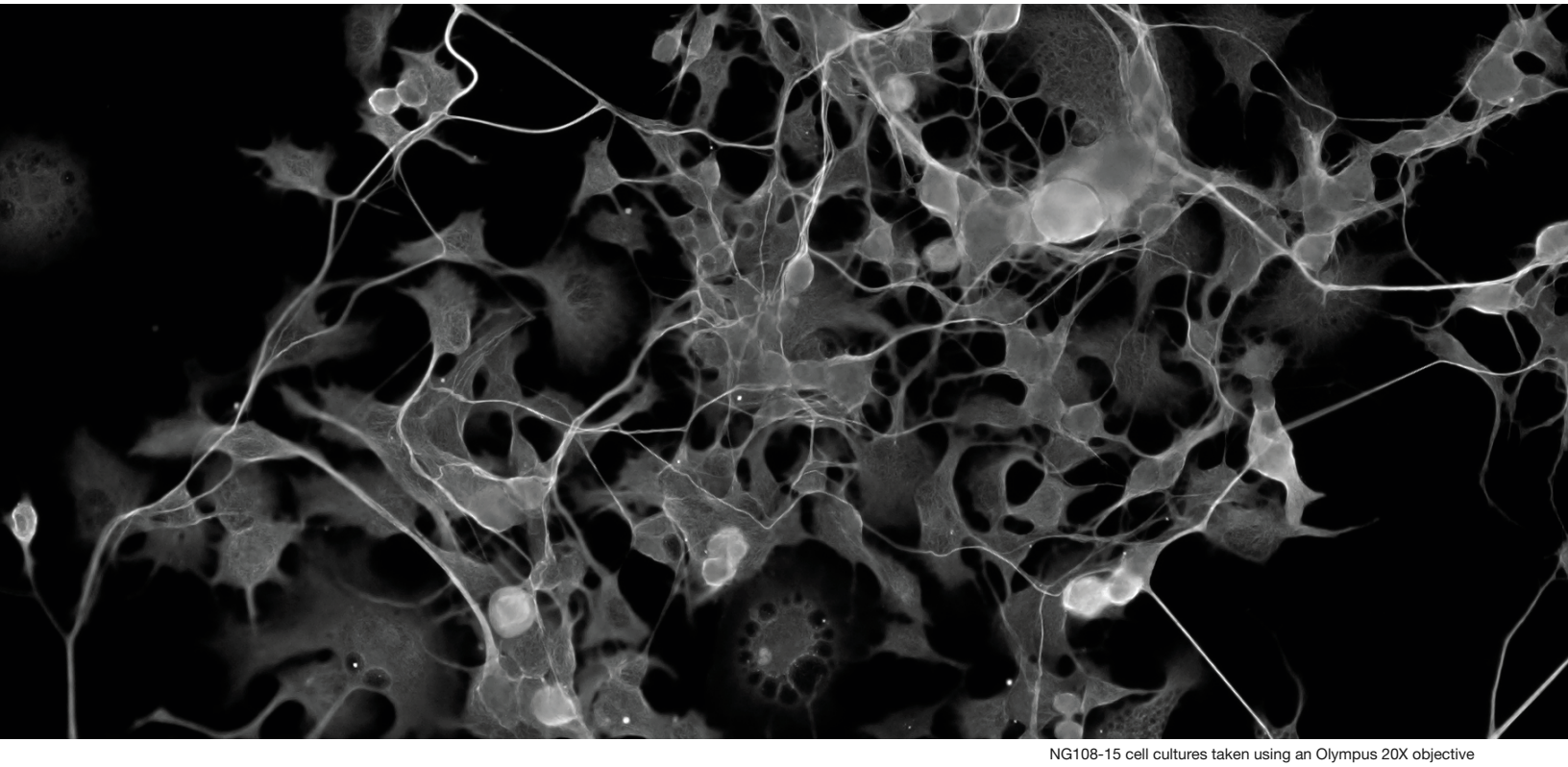

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# The Scientist TOP 10 INNOVATIONS

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- Products released on or after October 1, 2019 are eligible.
- Entries accepted from May 1 to August 21, 2020.

For further information, email: innovate2020@the-scientist.com

# Contributors

**Jennifer Smilowitz** was introduced to the concept of healthy living by her stepfather, a Russian physician who practiced holistic medicine. Accompanying him to international conferences as a child, she became "really interested in how the body works," and how you could "achieve optimal health through diet and lifestyle." As an undergraduate, Smilowitz decided that she didn't want to focus on diseases or treat patients with drugs, but instead hoped to prevent disease from ever arising. This choice led her to pursue her PhD in nutritional biology and a five-year postdoc in food science, both at the University of California, Davis. There she discovered that lactation was not only the "best model to understand food for health" but also to understand "how food actually delivers health in the body." In her current roles as associate director of the Foods for Health Institute and research scientist at UC Davis, Smilowitz has looked at a large cohort of mothers and babies from pregnancy through the first two years of the child's life to study how breast milk, complementary feeding, prebiotics, and probiotics influence the growth of beneficial microbes in the gut.

As a child, **Diana Hazard Taft** had a habit of blowing things up in the microwave. By the time she was in first grade, her parents were convinced she would become some kind of scientist. "I wanted to know why things did what they did," she says. Taft studied biology and chemistry at Cornell University, and then participated in the National Institutes of Health's Postbac IRTA program, focusing on the epigenetics of spermatogenesis and testicular cancer. She went on to complete a PhD in molecular epidemiology in 2014 at the University of Cincinnati, where she studied the microbiome of preterm infants for her dissertation. Now, as a postdoc at UC Davis, Taft researches full-term infant microbiomes and the genetics of lactation. She has come to understand "the host as part of the ecology of the microbiome, and the microbiome as part of the support for the host's health." In contrast to bacteria in the adult gut, which are more resistant to change, she says, "the infant microbiome is still a developing ecosystem, [and] small changes early on can have big implications later." On page 38 of this issue, Smilowitz and Taft write about how researchers might be able to take advantage of the malleability of the infant microbiome to develop probiotics that set babies on a healthy trajectory.

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**Neil Shubin** discovered his love for paleontology while interning at a dig site in Philadelphia during high school. "I was terrible," he remembers, but the act of doing science helped him find his calling. After majoring in biology and anthropology at Columbia University, he went on to Harvard University for a PhD in evolutionary biology. There, he saw a lecture on the "greatest hits" in the history of life, during which the professor showed a slide depicting what was then known about the transition of fish to land animals. Shubin recalls thinking, "That is a first-class scientific problem." To understand it would require new fossils as well as discoveries from embryology and ecology. It became the focus of his PhD research and the rest of his career.

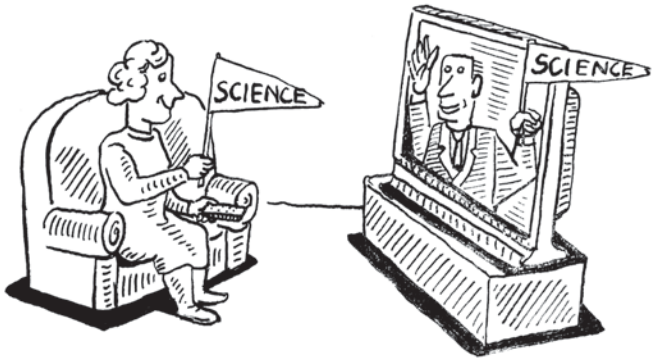
In 1993, Shubin discovered a roughly 365-million-year-old fossil from a species of hynerpeton, a genus containing some of the earliest creatures to walk on land, in a rock along the side of a Pennsylvania road. In the Canadian Arctic, he uncovered an even earlier fossil—some 375 million years old—of the ancient fish *Tiktaalik roseae*, which had webbed fins and bones that looked like structures that might be present in a cross between a fish and a tetrapod. Now a professor of organismal biology and anatomy at the University of Chicago, Shubin believes that we can use the tools of paleontology and developmental biology to explore the great transitions in evolution, from how animals came to walk on land to how birds began to fly. "Nothing ever begins when you think it does," he says, noting that lungs were present in fish before they left the water and that feathers appeared on dinosaurs long before creatures had wings. Read Shubin's essay, based on his latest book, *Some Assembly Required*, about how evolution repurposed existing structures to support major transitions in evolution on page 58.

ALANA JOLDERSMA; THE GOLDEN HOUR PHOTOGRAPHY; KELLI GOWDY PHOTOGRAPHY; JOHN WEINSTEIN, FIELD MUSEUM OF NATURAL HISTORY

# Armchair Scientists

Humility is key when thinking about and reacting to complex, unfamiliar situations.

BY BOB GRANT




ANDRZEJ KRAUZE

COVID-19 has caused disruptions large and small for months now. In late March, the International Olympic Committee postponed the start of the 2020 summer games in Tokyo, which was scheduled for this July. Among the many disturbances sparked by this worldwide disease outbreak, this one is not the gravest by far. But thinking about how I behave while watching the Olympic Games, summer or winter, I can't help but compare my behaviors to some of the reactions and opinions being shared by the public regarding coronavirus during this uncertain and unnerving time.

I don't consider myself a huge sports fan (though I can scarcely think of a more enjoyable way to fritter away a Saturday afternoon than lying on my sun-drenched couch nodding off in front of a televised baseball game). But when the Olympics are being broadcast, I transform into an armchair commentator, catching a full-blown case of what I like to call "Olympic fever." Biathlon, hammer throw, curling, Greco-Roman wrestling . . . whatever happens to catch my attention on any particular day, I submerse myself in the action until I actually start believing that I possess—and must share—some special insight into the sport. Never mind the fact that I know virtually nothing about the intricacies of hurling a giant steel object attached to a ropy tether through the air: "Ooh! He pulled his elbow in too early on that one. That's going to cost him." I've seen the same behavior in fellow spectators, and sharing this excitement and instant familiarity with a heretofore unfamiliar event is all part and parcel of enjoying the experience, in my opinion.

Unfortunately, I've noted a similar phenomenon in people living through the COVID-19 pandemic. And it's not so benign as feigning expertise in an arcane sport. Almost overnight, people who have had little exposure to epidemiology or virology—much less a formal background in these complex scientific disciplines—have what they present as well-formed and thoroughly researched opinions on the realities of this public health emergency.

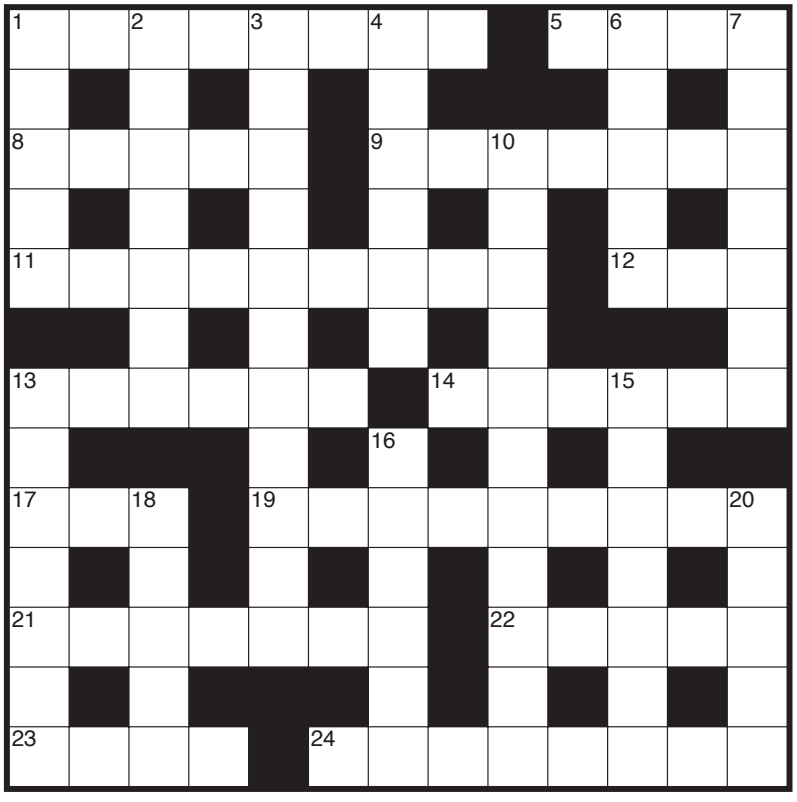
At least part of the blame for the recent proliferation of armchair experts is the raw amount of information at our fingertips. In a way, it makes sense that casual observers feel empowered and emboldened to opine on the scientific realities of such an important global event. People are scrambling to learn from myriad outlets everything they can about the virus and its spread. If every bit of news being broadcast were evidence-based and accurate, this smorgasbord of material would cause few problems. But sadly, we know this is not the case. It seems that this global emergency has worsened an already festering problem with information dissemination, mostly via the internet. Now more than ever, misinformation suffuses the stream of stories that we are absorbing. In a recent analysis published in *BMJ Global Health*, Canadian researchers reported that more than a quarter of the most viewed COVID-19 videos on YouTube contained "misleading information, reaching millions of viewers worldwide."

To be clear, I'm not suggesting that people should not have opinions about the course of events unfolding in our world or about the actions being taken by organizations, public health officials, or governments. On the contrary, they should. Diverse points of view brought to the table by people experiencing this pandemic within their own set of circumstances will help inform a variety of decisions. But as we battle SARS-CoV-2, science, evidence, and reason are our sharpest weapons. And the most responsible attitude for the nonscientist (and the noneconomist, for that matter) to adopt is one of humility and deference. Being interested in and following along with the scientific details of this pandemic as researchers and other experts learn more about the pathogen and how people are affected by it is one thing. Forming a calcified stance on how and when corrective actions should be taken based on a cursory and hastily assembled understanding of the biology or economics involved in this complex and difficult situation is quite another.

Unfortunately, the new reality of mis- and disinformation is something our global society will live with now and for years to come. We at *The Scientist*, along with our colleagues in science journalism, will continue to combat this foe by trying to engage people's attention with accurate and data-driven stories. In the meantime, everyone can work to stem the tide of faulty logic, conspiracy theories, and intentional obfuscation by invoking a phrase that has become all too rare in our information age: "I don't know." That may be a hard sentiment to conjure in an era where most answers come literally at the click of a button and when uncertainty can be as terrifying as the microbial threat we face. But now, more than ever, it is crucial that we respect the hard-won experience and knowledge of scientists and public health experts, supporting their efforts and valuing their advice with the trust they deserve. ■

Editor-in-Chief
eic@the-scientist.com

# Speaking of Science



Note: The answer grid will include every letter of the alphabet.

BY EMILY COX AND HENRY RATHVON

## ACROSS

1. Site of many a cryptozoological hunt (2 wds.)
5. Any pathogen, informally
8. Young nesting in a barn, perhaps
9. Anatomical blade
11. Herb used in tea and gum
12. "Bad" cholesterol, for short
13. Film of a molecular-transporter mishap (2 wds.)
14. Units of energy
17. Colorful fish in a pond or water garden
19. Extant but nonfunctional
21. Common name for one *Danaus* butterfly
22. Cessation of breathing
23. Robert who espoused the 5-Across theory of disease
24. Group of one-celled eukaryotes

## DOWN

1. Focus of study for zoologist Craig Packer
2. Where Thomas Edison never went
3. Trail walker with a field guide, say (2 wds.)
4. Pine ___ (kin to a goldfinch)
6. Like watt-seconds and 14-Across
7. Illness identified with a spot check?
10. Substance called a "free-radical scavenger"
13. Experimental fusion reactor of Russian invention
15. German polymath who codeveloped calculus
16. Relativity lithographer
18. Pertaining to charged particles
20. Camel's Andean cousin

*Answer key on page 5*

You know, the conspiracy theories out there have essentially closed down communication between scientists in China and scientists in the US. We need that communication in an outbreak to learn from them how they control it so we can control it better. It's sad to say, but it will probably cost lives.

—**Peter Daszak,** president of the nonprofit EcoHealth Alliance, talking to "60 Minutes" about the ongoing politicization of science with regard to the COVID-19 pandemic (May 10)

The major message that I wish to convey . . . is the danger of trying to open the country prematurely. If we skip over the checkpoints in the guidelines to: "Open America Again," then we risk the danger of multiple outbreaks throughout the country. This will not only result in needless suffering and death, but would actually set us back on our quest to return to normal.

—Infectious disease researcher **Anthony Fauci**, head of the National Institute of Allergy and Infectious Diseases and a leading member of the Trump Administration's White House Coronavirus Task Force, in an email to *The New York Times* reporter Sheryl Gay Stolberg about his plans to testify before the Senate (May 11)

JONNY HAWKINS

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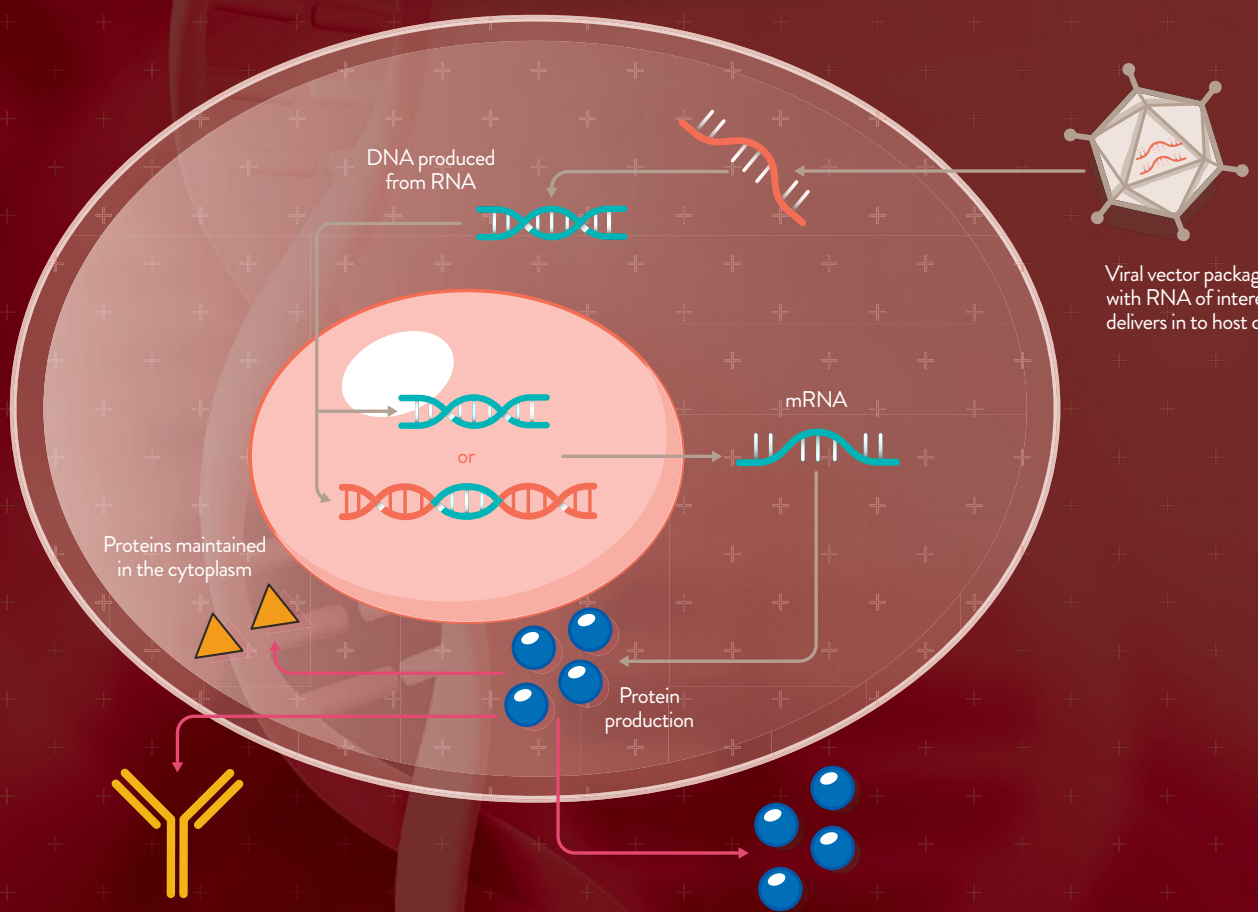
# TRANSDUCTION

Transduction is the process of using vectors including retroviruses, lentiviruses, adenoviruses, adeno-associated viruses, or hybrids to deliver genetic payloads into cells. Generally, a plasmid carrying genes flanked by viral sequences is first transfected into a producer cell with other virus-associated (packaging) plasmids. In the producer cells, virions form that contain the gene of interest. For safety, no plasmid used in the process contains all of the necessary sequences for virion formation, and only the plasmid carrying the gene of interest contains signals that allow it to be packaged into virions. Researchers then extract, purify, and use the virions from the producer cells to insert DNA into other cells to stably or transiently express the DNA of interest. The transferred genetic material, which lacks viral genes, cannot generate new viruses.

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# PACKAGE DELIVERY:
# The Art of Transfection

Inserting genetic material into mammalian and insect cells without killing them can be a challenge, but scientists have developed several ways to perform this intricate task. Transfection is the process of introducing nucleic acids (plasmid DNA or messenger, short interfering, or micro RNA) into a cell. Researchers accomplish this with nonviral methods (chemical or physical transfection), or with viral methods, commonly referred to as transduction.

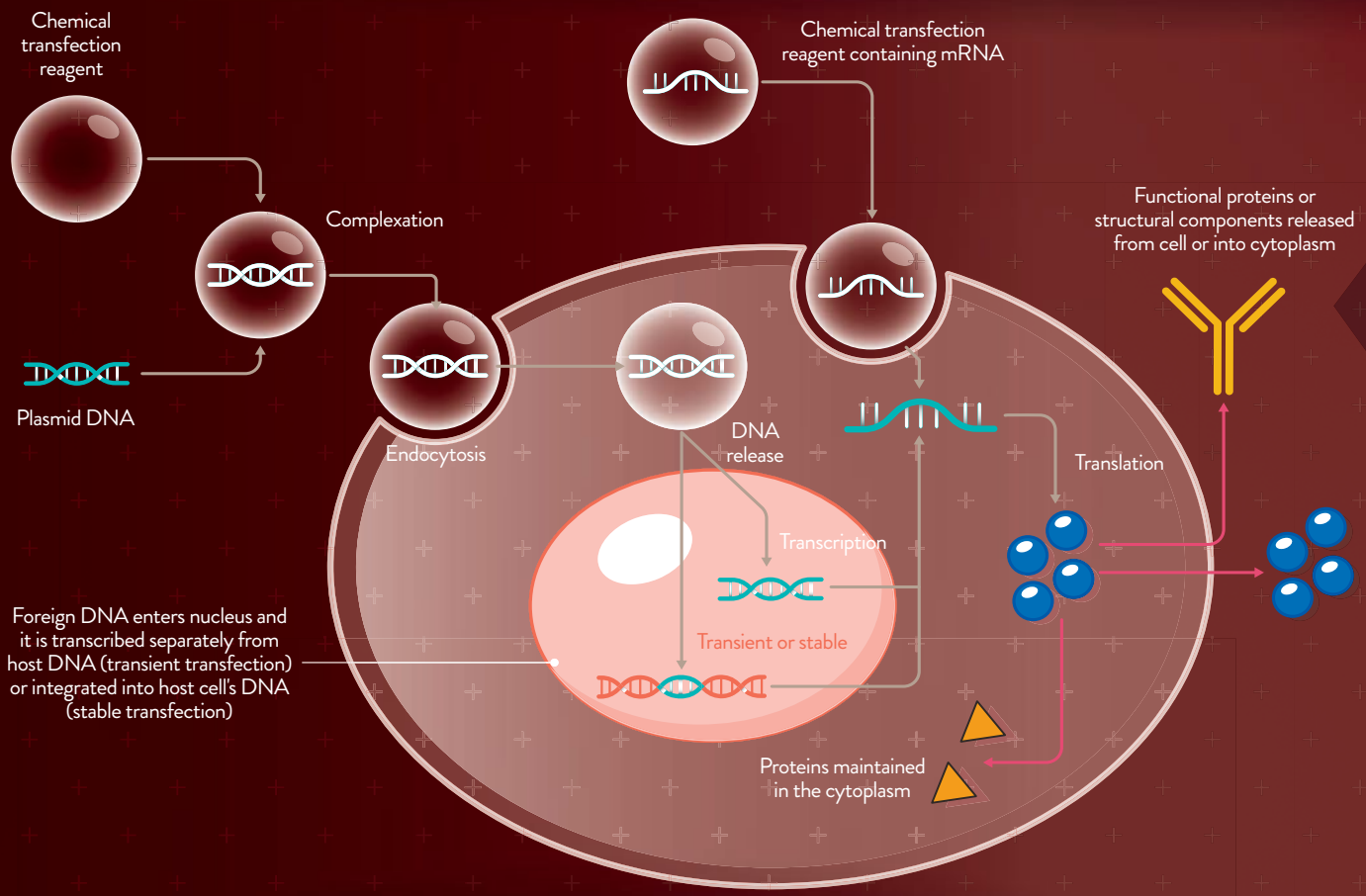
Researchers use chemical and physical transfection and viral transduction to explore gene expression and screening, for bioproduction of proteins and viruses, or for therapeutic purposes such as gene therapy. Successful nucleic acid delivery depends on the quality of DNA, ratio of DNA to a chemical reagent, cell passage number and confluency, and post-transfection incubation time. Non-viral transfection is commonly used for adherent immortalized cells, while transduction is often employed for the most difficult cell types, including primary, stem and immune cells. Transfection sometimes improves outcomes when using large DNA inserts because viral vectors have an insert limit of ~4.5 kb (adenoviral-associated vectors) to ~10 kb (lentiviral vectors).

## TRANSIENT VS. STABLE TRANSFECTION

DNA transfection can be classed as stable—where the foreign gene integrates into the host genome—or transient—where the gene does not integrate into the genome. Transfection leads to transient or stable expression of DNA in cells, depending on the method or the viral tool used. Transfection of RNA, however, is always transient. Whether a scientist uses stable or transient transfection depends on the desired experimental outcome.

| TRANSIENT | STABLE |
|---|---|
| Foreign DNA does not integrate into the host genome | Foreign DNA integrates into the host genome |
| Short-term expression | Long-term expression |
| Usually chemical or electroporation-based | Usually involves viral vectors or targeted endonucleases |
| No potential for non-specific integration | Potential for non-specific integration |
| Useful for manipulating specific gene activity in cell culture | Useful for gain- or loss-of-function studies |
| GFP or other co-expressed markers can be used to check for successful transfection/transduction | Co-expressed selection markers can be used to produce a selectable advantage, or lentiviral vectors can insert DNA randomly into the host genome |

# NON-VIRAL TRANSFECTION

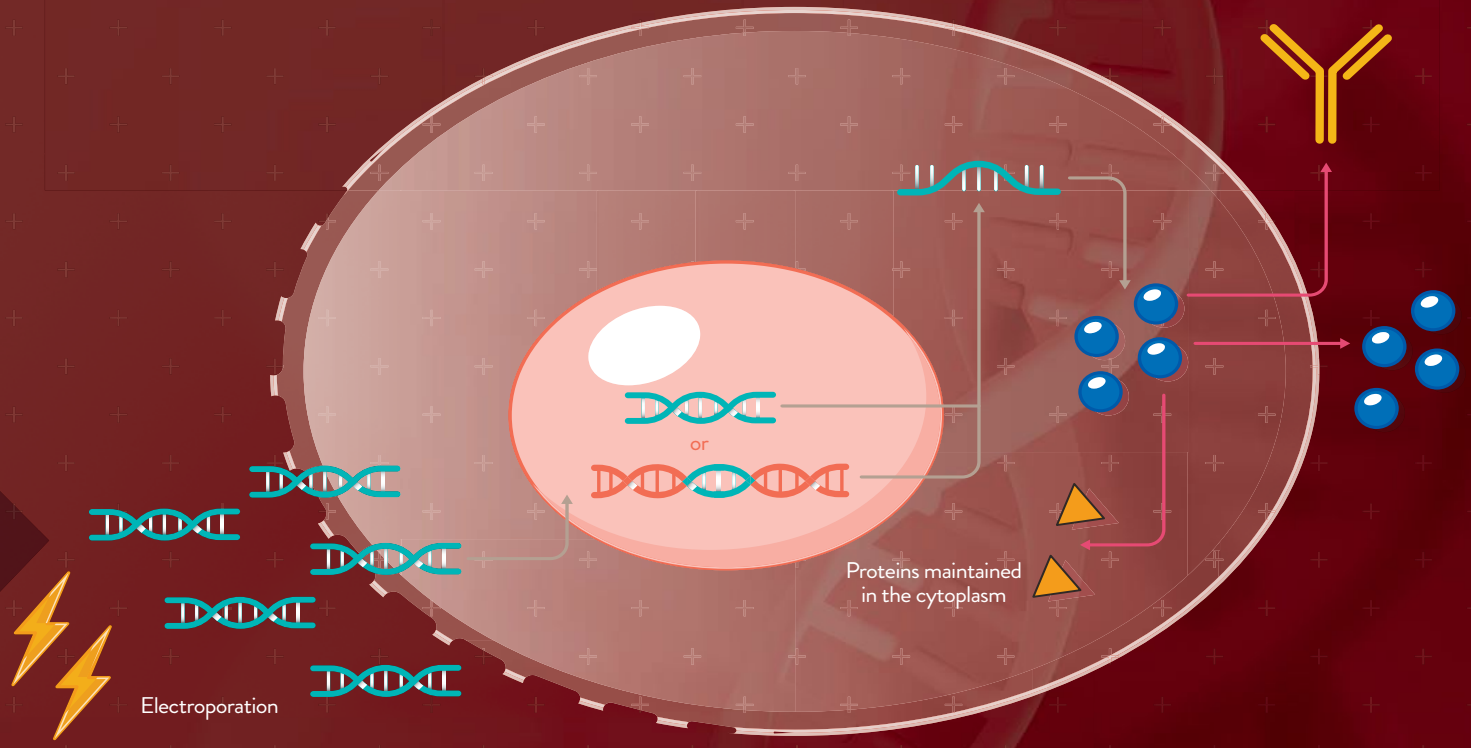



## ELECTROPORATION

Electroporation is the most common physical transfection method and is often used for primary, progenitor, and stem cells. Electroporation causes cell membrane permeability via short pulses of an intense electric field. During the pulse, temporary physical channels are created in the membrane that allow the desired cargo to enter the cells. Electroporation is easy, quick, and reliable, but can be stressful for cells, rendering the techniques unsuitable for difficult-to-culture or sensitive cells due to high cell-death numbers. One of the primary advantages of electroporation is its versatility—it can be adapted to deliver diverse types of nucleic acids and other molecules to virtually any mammalian cell type.

## CHEMICAL TRANSFECTION

Chemical carriers represent the most straightforward and widespread tools for gene delivery experiments in mammalian cells. Chemical transfection experiments follow a simple workflow and provide high efficiency nucleic acid delivery for the most commonly used cells as well as many hard-to-transfect cell lines. One of the oldest chemical transfection approaches was the use of calcium phosphate, which in the hands of a skilled technician, could be used to co-precipitate DNA for subsequent delivery to certain permissible cell types. More modern approaches achieve higher transfection efficiencies in a broad array of cell types and utilize proprietary commercial lipids and polymers that are precisely engineered for optimal DNA condensation, electrostatic interaction with target cells, cellular uptake and endosomal release.

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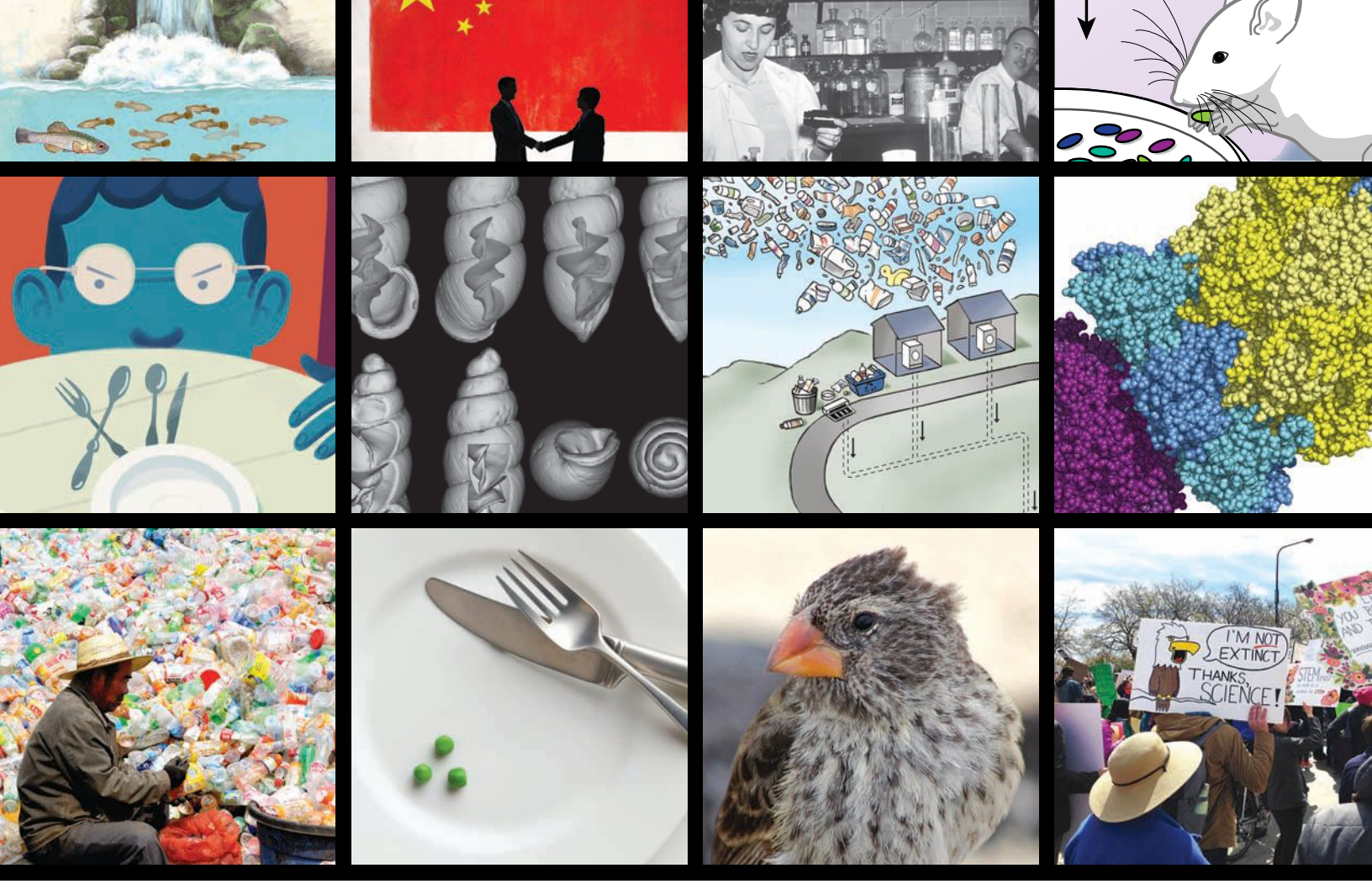
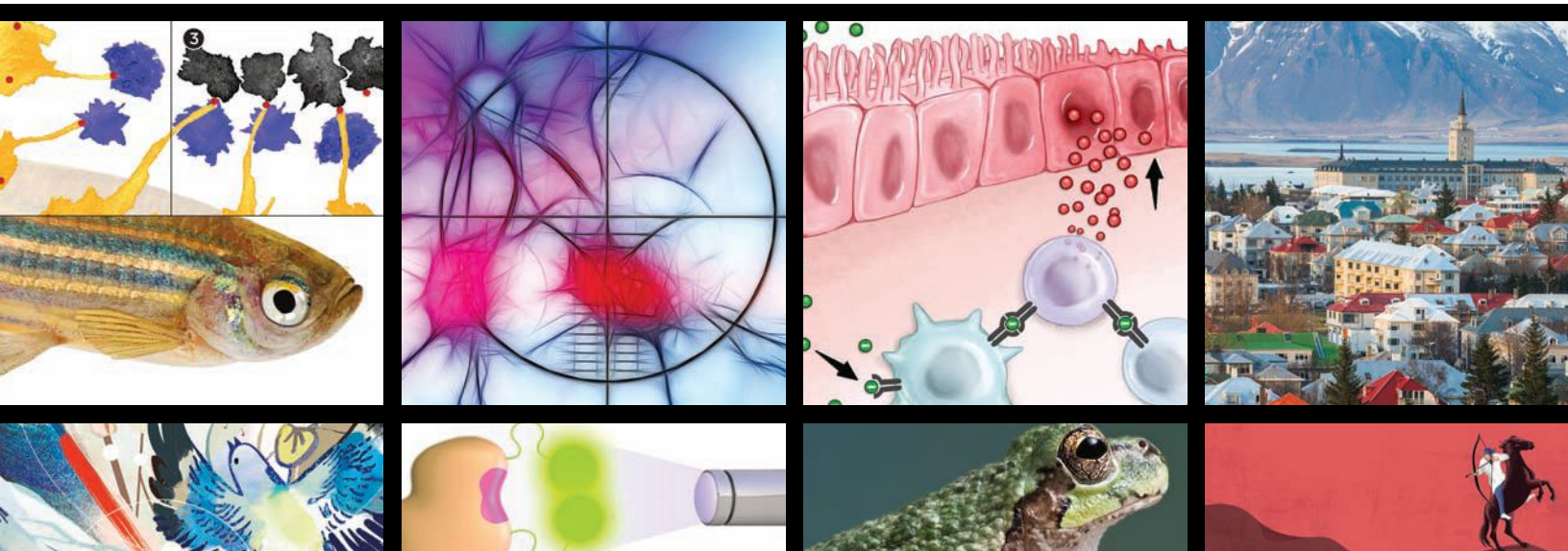




# Notebook

JUNE 2020

WIKIMEDIA, MARCELLO CASAL JR/AGÊNCIA BRASIL

**PERFECT STORM:** Droughts in northeastern Brazil indirectly led to increased incidence of microcephaly in babies born during the Zika epidemic, a study suggests.

## Confounding Factor

For four years starting in 2012, dry weather crackled across northeastern Brazil. Temperatures rose, vegetation died, and fresh water started to evaporate more quickly from the region's reservoirs. Though the conditions were devastating for local human populations, they were ripe for bacterial blooms to flourish in the water. Among the bacterial species known to establish themselves in reservoirs and wells during such droughts is the cyanobacterium *Raphidiopsis raciborskii*.

More often than not, the strain of *R. raciborskii* that colonizes reservoirs and wells in the region is one that secretes saxitoxin, a chemical that lets the bacterium thrive in the salty, mineral-rich water associated with dry spells. Although saxitoxin aids the bacterium's survival, it is—as its name indicates—toxic to humans, who often ingest it when eating contaminated freshwater shellfish. In large amounts, the neurotoxin can be deadly, causing respiratory failure; in lesser amounts, it leads to numbness and partial paralysis.

Because of this toxicity, Brazilian water quality guidelines state that levels of saxitoxin must be lower than three micrograms per liter, which would keep people safe if they consume the contaminated water only infrequently. During droughts, however, that water is likely to be more contaminated than normal, putting people at risk of higher exposure to saxitoxin.

To explore the effects on the brain of regularly drinking contaminated water, Katie O'Neill of the University of Adelaide and colleagues set up a lab experiment with cultured nerve cells in 2016. Continuous, low-level exposure to saxitoxin impaired the cells' growth, the team found, making it hard for them to form the spiny protrusions that are essential for cell-to-cell communication. The neurotoxin also disrupted expression of proteins involved in mitochondrial function and in programmed cell death, the team found (*Basic Clin Pharmacol Toxicol,* 120:390–

97, 2017). It was a hint that, even in low doses, saxitoxin may pose a risk of neurological damage.

As the dry spell lingered into the 2010s, northeast Brazil was hit with a second health crisis: an outbreak of illness spread by mosquitoes infected with the Zika virus. Zika swept across South America and other regions of the world in 2015. As it did so, doctors treating patients sickened by the disease began to note that pregnant mothers who gave birth after being infected sometimes had babies with microcephaly, a condition in which the baby's head, and often also its brain, are much smaller than normal.

"Northeast Brazil was the epicenter for cases of microcephaly," says Stevens Rehen, a neuroscientist at the Federal University of Rio de Janeiro. "But the incidence of Zika was higher in other regions of Brazil." That discrepancy led Rehen and his colleagues to wonder if some environmental factor was compounding the effects of viral infection in pregnant women in the northeast region, leading to more-severe brain damage in their babies. When his team saw that northeast Brazil suffered its worst drought on record at the same time as the Zika outbreak, the researchers decided to test whether saxitoxin and Zika together spurred serious changes to brain tissue.

The team started tests in human brain organoids, growing clumps of nerve cells from the reprogrammed skin cells of donors. After cultivating the cells for 50 days, Rehen and his colleagues infected the brain organoids with Zika virus and then treated them daily with low doses of saxitoxin. After 13 days of treatment, the team looked at the organoids under the microscope and found that the clumps of brain cells exposed to both Zika and saxitoxin had 2.5 times more dead cells than organoids that were only infected with Zika. Organoids exposed to both saxitoxin and Zika also had levels of the virus that were three times higher than what was found in Zika-only cultures, suggesting that the toxin promoted viral replication. Organoids treated only with saxitoxin had a level of cell death similar to what was observed in untreated organoids.

**Northeast Brazil was the epicenter for cases of microcephaly. But the incidence of Zika was higher in other regions of Brazil.**

—Stevens Rehen, Federal University of Rio de Janeiro

Wanting to check the results in animals, Rehen and his colleagues set up an experiment in which female mice were given either clean or saxitoxin-contaminated drinking water for a few days before and a few days after mating. After the mice became pregnant, the researchers infected them with Zika. Baby mice born to Zika-only mothers had fairly normal brains, the team found, but those born to Zika-infected mice drinking saxitoxin-laden water had unusually small brains, with around a 30 percent reduction in the thickness of their cortex, a layer of the brain known to be essential for cognition. Those mice also had twice the number of dead nerve cells in their brains as the pups whose moms were only infected with Zika or were not subjected to either treatment, the researchers reported earlier this year (*PLOS Negl Trop Dis*, 14:e0008060, 2020).

"This paper demonstrates that a long-standing problem with cyanobacteria toxins in the water resources of the region has played a role in making the impact of the Zika outbreak in the region much worse," says Alexandre Anesio, a biogeochemist at Aarhus University in Denmark who was not involved in the work.

Rehen notes that the research not only shows a connection between saxitoxin and Zika, but also exposes a potential reason for the observed economic disparity in severity of illness. Initially, "we were surprised by the fact that many babies with microcephaly were born at Brazilian cities with very low gross domestic product," he writes in an email to *The Scientist*. In light of his team's findings, it seems that "unfortunately, these malformations were probably exacerbated by avoidable environmental cofactors associated with poverty and lack of basic needs."

The importance of improving access to clean water is clearly shown in this new work, notes Fabiano Thompson, a microbiologist at the Federal University of Rio de Janeiro who was not involved in the study. "Even in countries like Brazil, which has 10 percent of the fresh water of the planet, water is a pricey resource," he says. "Governments need to be very careful with this."

—Ashley Yeager

## Listening in on the Birds

It turns out that pulling a wagon laden with cat litter buckets and speakers around a small college town in the dead of winter can invite questions from strangers. When Oberlin College undergraduate Abby Parker explains to curious residents that she's doing research on squirrels, she says, the response is often, "Squirrels? Plenty of those around here!"

Parker's goal is to find out whether Eastern gray squirrels (*Sciurus carolinensis*) respond differently to the chatter of familiar North American birds than to unfamiliar species. Finding the answer will help identify what types of information the animals glean from other species' utterances.

When Parker or one of the other students involved in the study finds a squirrel, they set up the two speakers 4 meters apart atop overturned Tidy Cat buckets at least 10 meters from their subject. They wait a minute to give the animal a chance to acclimate to the presence of the gear and its human operator, then record the animal's activities for 30 seconds via a custom-made phone app. Next, they play a red-tailed hawk call through the speakers to put the squirrel on alert for a predator, and observe the animal's behavior for another 30 seconds. Finally, they play one of another four sounds: ambient noise; the chatter of familiar, North American bird species; the chatter of Australian birds that the squirrels have presumably never heard before; or song from these

same Australian bird species. They watch the squirrel carefully for another three minutes to see how it behaves. Does it groom itself? Forage? Perhaps most tellingly, how frequently does it look up from what it's doing to check for predators?

The study, overseen by Oberlin biologist Keith Tarvin, is a follow-up to one conducted with his former student Marie Lilly, now a graduate student at San Francisco State University. Tarvin, Lilly, and another student found that after hearing a recorded hawk call, squirrels that subsequently heard the normal chatter of familiar bird species displayed fewer vigilance behaviors, such as freezing or looking up, than did squirrels that heard bird-free ambient noise.

**These squirrels are much more aware of their surroundings than I think most people would have given them credit for.**

—Mike Webster, Cornell Lab of Ornithology

Those findings, the authors wrote in their paper, suggest that the familiar chatter served as an auditory signal that the surroundings were safe again (*PLOS ONE*, 14:e0221279, 2019). Animals are known to be attuned to birds' alarm calls, Lilly says, so it made sense they might also pay attention to normal chatter. Not only did the data bear that out, but "it was definitely a lot stronger of results than I was anticipating, which was really cool," she says.

The question now, Tarvin says, is whether the squirrels are indiscriminate in their eavesdropping—that is, if they've learned "that the presence of a soft din of chips, chortles, buzzes, and the like tends to be associated with safety"—or if they are responding to the calls of specific species. "It certainly is possible that squirrels also learn to recognize and interpret a wide array of [non-alarm] calls from a host of disparate species, but that seems to require a whole lot of learning on the part of the squirrels, and a lot of processing too," he notes. If they do devote this much brain power to bird calls, it could indicate that they're gathering richer information than simply danger or safety. The current study is a step toward answering that question.

Tarvin's other research projects have mainly been on the behavior of birds themselves. He only started studying squirrels after finding a squirrel observation activity in an instructor's manual and using it in one of the courses he teaches. He later realized that, as wildlife that are abundant and acclimated to human presence in Oberlin, squirrels would be an ideal subject for undergraduate research projects on animal behavior.

Designing and conducting experiments on squirrels is no walk in the park, however. One abandoned experimental design involved playing a sound in an area with squirrels in the morning, then returning to observe their behavior later in the day (with the assumption that because squirrels have small territories, the animals present in the afternoon would likely have heard the sound in the morning). But it was rare to spot any squirrels at all on the follow-up trip, and on one of the few trials where Tarvin's student did find one, a hawk swooped down and nabbed it just as the researcher was conducting his observation.

The fact that squirrels are indistinguishable from one another even for trained observers causes complications as well. To avoid observing the same animal twice, Parker and the two other students involved in the current study track the locations where they've played sounds on a shared Google map and avoid returning to those areas for trials for at least a few weeks. Trials are sometimes ruined because the animal under observation runs behind a tree and emerges with an identical companion or two.

While animal responses to alarm calls have been well-studied, the paper the group published last year is unusual in that it gets at the other side of those behaviors, says Erick Greene, a behavioral ecologist at the University of Montana who was not

ANDRZEJ KRAUZE

involved in the study. "One of the things that's not been as clear . . . is how animals know that it's okay to go back to sort of normal life," he notes. "And this is an important point because if you, say, go back to foraging too early and there still is danger around, you could get nailed and get killed by a predator." The authors of the 2019 study, he says, "really hit it out of the park and addressed a really interesting question that not many people have looked at."

Mike Webster, who has collaborated with Tarvin in the past and now studies animal communication and behavior at the Cornell Lab of Ornithology, agrees that the results published so far are a valuable contribution to understanding how animals gather information from sound. "These squirrels are using this public information that's available to them in the outside world in a way that we didn't really understand before, and they're much more aware of their surroundings than I think most people would have given them credit [for]," he says. One question the study raises, he notes, is: "Are other species doing the same thing?" And if so, is the behavior limited to mammals, or might birds, reptiles, and other taxa also rely on such public information to assess safety? Webster also wonders whether animals could derive different types of information—about suitable spots for foraging or nesting, for example—from other species' auditory signals.

Unfortunately, the answers to such follow-up questions, including those posed by the group's current study on Australian versus native bird sounds, will have to wait. In mid-March, the month after that study began, the Oberlin campus shut down due to the COVID-19 pandemic, sending students home for the rest of the semester. Given that the trees will have leafed out by summer, obscuring potential study subjects from view, Tarvin expects that the study won't resume until winter sets in. "Abby took a set of gear home with her in hopes of being able to do some trials in Pennsylvania," he writes in an email to *The Scientist*, "but given the transitions to online courses and other disruptions, I don't know if she'll have time to do much squirrel work."

—Shawna Williams

# Between the Legs

Last summer, evolutionary biologist Xavier Zahnle invited fellow millipede researchers to a visual treat. A PhD student at the University of California, Davis, Zahnle had just produced digital images showing the rarely seen insides of the male sexual organ, or gonopod, of the millipede *Pseudopolydesmus serratus*. Zahnle's collaborators eyed the images carefully, looking for answers to the decades-old question of how millipede males deliver sperm.

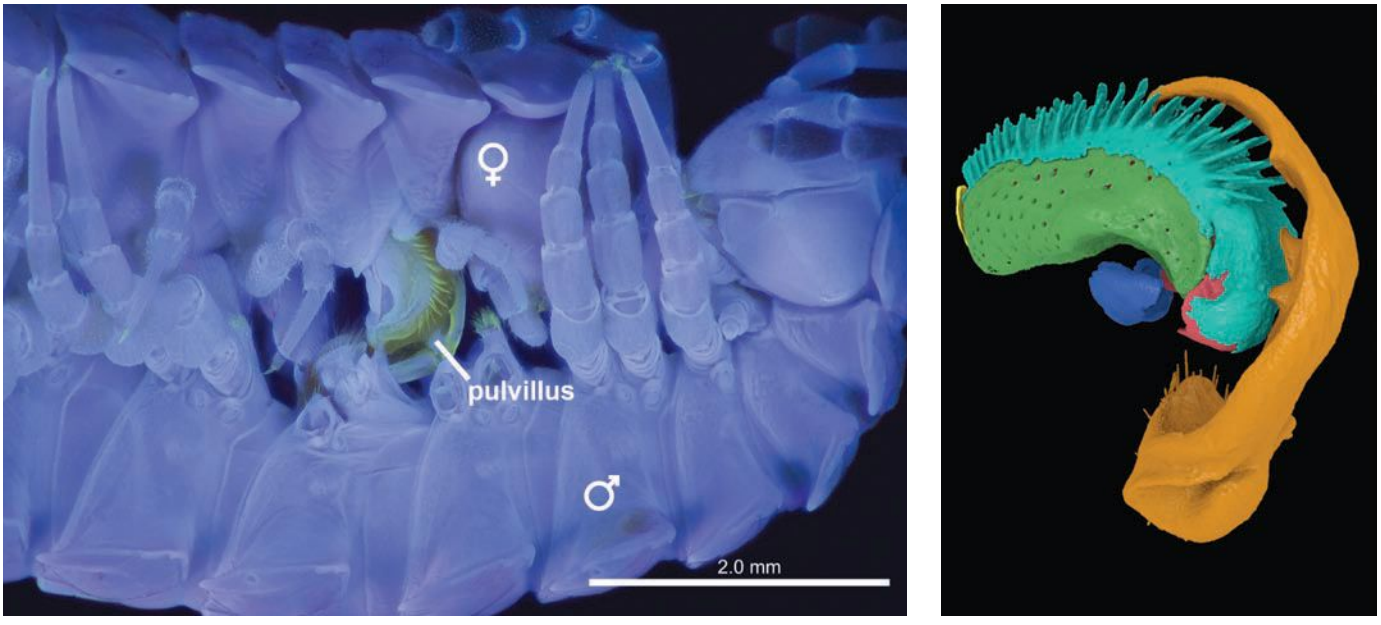
Millipede sex is a shrouded affair that happens behind rows of legs. A female millipede's sexual organs—a pair of vulvae—are located in the third body segment from the head. When she is ready to mate, she pushes her vulvae out from behind her legs. The male grabs her with his many legs, then clasps her vulvae with his two gonopods, each just a millimeter long, and inserts a hairy stub on the surface of a gonopod into the female. Since 1931, millipede researchers have suspected that a tube called a seminal canal in the gonopod carries sperm directly out of this stub and into the female. In a 2019 review paper of the genus *Pseudopolydesmus*, Zahnle and his colleagues supported the same idea. But nobody had had the opportunity to verify this—until now.

To get a closer look, Zahnle, along with Petra Sierwald, an associate curator of arachnids and myriapods at the Field Museum of Natural History in Chicago, and other colleagues, had turned to state-of-the-art imaging techniques, including scanning electron microscopy and ultraviolet fluorescence imaging. Applying these techniques on specimens from the collections of the Field Museum, including a pair of *P. serratus* preserved while copulating, the team reconstructed how millipedes mate.

They then looked inside the animals using an imaging technique called micro–computed tomography, or micro-CT, which shoots X-rays at a specimen to produce a series of grayscale images in which denser parts in the specimen show up brighter. A computer program compiles these images into a block of pixels, and the human user directs the software to color the different structures in the block to produce a 3-D replica of the specimen. Researchers can study such digital models of specimens "from every angle," says Zahnle. "You really, really, really get a sense of how things are oriented, and what's next to what."

Zahnle's micro-CT images revealed a surprising twist in how a male millipede delivers sperm. Rather than confirm that the seminal canal leads directly to the hairy stub outlet, the images showed instead that the canal bypasses the hairy stub and sends the sperm into a chamber. Only then do the sperm exit through the hairy stub. The canal "makes a complete loop, which is totally mental," says Zahnle, and "not at all what we described in our [2019] paper. We were wrong." He and his colleagues published their results in *Arthropod Structure and Development* earlier this year (54:100913).

Javier Alba-Tercedor, an aquatic entomologist at the University of Granada in Spain who was not involved in the work, says Zahnle's study "is a good example of what can be achieved by using micro–computed tomography." Alba-Tercedor, who has won awards for his micro-CT




**INTIMATE VIEW:** Researchers at the University of California, Davis, use multiple imaging techniques to study millipedes. The ultraviolet-enhanced image (left) shows two *Pseudopolydesmus serratus* preserved in mating position. The 3-D model (right) was created using micro-CT imaging and shows genitalia of the female (green-turquoise) and male (orange).

STEPHANIE WARE; XAVIER ZAHNLE

**You really, really, really get a sense of how things are oriented, and what's next to what.**

—Xavier Zahnle,
University of California, Davis

work, says the most time-consuming part of the technique is coloring the different structures in the images. But once that is completed, the datasets can be used by other researchers to study new structures in the specimens.

Indeed, Sierwald sees micro-CT as a game-changer for millipede studies. When she started studying millipedes in 1997, micro-CT was rarely, if ever, used in entomology. Her initial plan to examine female millipedes' vulvae from the animals' discarded molts failed because the "darn millipedes eat their molts." Without micro-CT, "it would have meant killing a lot of millipedes in order to dissect them," she says. With micro-CT however, no dissections are necessary, meaning that fewer animals are killed and those that are stay intact for future research, "so that's really cool." She adds that micro-CT helps to "really make a big step forward" in understanding how the sexual organs function together.

In the long run, Sierwald says, the team hopes that the imaging methods will help researchers identify millipede species, monitor their geographic distribution, and learn more about how different taxa are related to one another. She also suspects that the secretions that millipedes produce during sex might have commercial or pharmaceutical uses.

Thomas Simonsen, an entomological researcher and curator at the Natural History Museum Aarhus in Denmark who was not involved in the research, says that the new study is "excellent," and agrees with Sierwald that the methods could aid millipede research. He notes that, along with DNA studies, it's important to have detailed knowledge of morphology to "understand the diversity and evolution of arthropods," especially in taxonomic groups for which phylogenetic relationships are poorly understood.

Zahnle is focusing on male millipedes now. To him, millipedes display myriad forms and functions. They're not "just lots of legs," he says, but range from "flat-back to large cylinders to tiny bristles." After a steep learning curve with micro-CT, Zahnle finds himself in "good company" among the growing number of arthropod researchers using the technique. He wants to know if the unexpected loop in the sperm canal of *P. serratus* shows up in other species, he says. "I want to image them all."

—Yao-Hua Law

# Stool Standards

The first thing Martha Carlin noticed was a faraway look in her husband's eyes. It was a subtle change, she says, something only a wife would see. She happened to be reading *Lucky Man* by Michael J. Fox at the time, and began to wonder about some of the symptoms she'd observed in her husband: his loss of facial expressions, his quivering pinky finger, his trembling tongue. An appointment with an internist led to an appointment with a neurologist, who confirmed Carlin's worst fears. In the fall of 2002, her husband John was diagnosed with Parkinson's disease. He was 44 years old.

Carlin spent the next seven years reading everything she could find on Parkinson's. A consultant skilled at identifying breakpoints in businesses, Carlin concluded that the disease is "a systems problem," she says, a collapse of the body's ecosystem. Much of what she had learned implicated the gut, including the finding that constipation is one of the earliest symptoms of Parkinson's, often emerging 10 years or more before a diagnosis. In late 2014, Carlin read a study that identified a specific imbalance in the composition of Parkinson's patients' gut microbiomes, suggesting that changes in the gut microbiota could be an important biomarker for the disease. "That is it," she remembers thinking. "The gut is the general ledger of the body."

She quit her consulting job and enlisted the help of Jack Gilbert, a microbiome expert then at the University of Chicago. Carlin paid Gilbert, who serves on *The Scientist*'s editorial advisory board, to analyze her and her husband's stool samples, and also donated $30,000 to cover part of the salary of one of his postdocs. Together, she and Gilbert pored over the microbiome literature. They kept coming across mentions of certain microbial genes that were overexpressed in conditions such as Parkinson's and autism, suggesting "functional similarities at a systems level," Carlin says. It seemed logical to her that investigating such complex conditions would require looking at the whole community of bacteria in the living system.

At the time, however, microbiologists weren't really focusing on whole microbial communities, says Carlin. Instead, most research involved taking small samples of stool and analyzing 16S ribosomal RNA. She and her collaborators were convinced that stool held much more information than one small scoop could capture. They proposed collecting the whole stool, homogenizing it, and cataloging all the bacteria in the sample using whole-genome sequencing. That approach, Carlin says, yields 2–8 gigabytes of genetic data per sample, compared to the few kilobytes produced with 16S sequencing. There wasn't any company set up to process stool in this way, so she decided to start her own.

Carlin founded The BioCollective in 2015, and within a year she and her team had designed and patented the BioCollector Kit, an "ick-free" paper hammock that catches the entire stool, which is then shipped to the lab, homogenized, and aliquoted into identical portions. Aided by publicity from a handful of bloggers, the team collected samples from hundreds of people. Carlin also targeted Parkinson's support groups. Researchers soon asked to use the kits, too—among them scientists at the Dana-Farber Cancer Institute and San Francisco–based biotech company Siolta Therapeutics.

For microbiologists, the appeal of The BioCollective's approach lies in both its easy collection method and its potential to standardize the field by providing a reliable control against which to compare other human gut microbiome samples. Microbiome research suffers from a high degree of variability, says Scott Jackson, a biochemist who leads the Complex Microbial Systems Group at the National Institute of Standards and Technology (NIST) and partners with The BioCollective. Variation in methodology crops up everywhere, "from how you extract DNA to how you build your [DNA] library" he says. Two different labs analyzing the same stool sample will often get "very different results." If researchers could agree on a single reference standard, with a known taxonomic composition, he argues, they would be able to "understand reproducibility—or the lack thereof—across laboratories."

NEW APPROACH: In 2015, Martha Carlin founded The BioCollective and began work on a stool-collecting kit that could help microbiome researchers standardize their work.

Jonathan Jacobs, a gastroenterologist and microbiome researcher at the University of California, Los Angeles, who has not collaborated with Carlin or The BioCollective, agrees that a "reference standard would be helpful in assessing the variation across different labs and for the labs to monitor technical changes over time in their own pipelines."

As Carlin and her team continued to develop TruMatrix, their signature reference standard, they were simultaneously studying samples in their bank, which holds stools from nearly 1,000 people, including some multi-year samples in the Parkinson's cohort. Her team has created a computational model called BioFlux to predict how gut bacteria will react to various chemicals. Carlin says the model could be used to study how pharmaceuticals, nutraceuticals, and different foods influence the microbiome of Parkinson's patients.

ADAM BEACH PHOTOGRAPHY

**Carlin and her collaborators were convinced that stool held much more information than one small scoop could capture.**

In early 2018, the company released SugarBuster, a probiotic that The BioCollective notes may help boost gastrointestinal concentrations of the sugar alcohol mannitol. Some research has suggested that mannitol can inhibit the formation of amyloid fibrils, an early sign of Parkinson's, in a *Drosophila* model of the disease. The probiotic has not been evaluated in clinical trials, although Carlin says the team is planning a trial in diabetes patients later this year. And in February 2020, The BioCollective was awarded a $1.2 million Fast Track Grant from the National Institute of General Medical Sciences (NIGMS) to develop the first national microbiome reference standard.

But not everyone thinks that standard reference material for the human microbiome will impact microbiological research. Mayo Clinic gastrointestinal researcher Purna Kashyap, who has not collaborated with The BioCollective, cautions that this reference will need "a buy-in from the microbiome community." He adds that he would be more convinced of its value if Carlin's team were to conduct research showing that the company's reference microbiome, which contains hundreds if not thousands of different bacteria, yields results that contradict previous studies when used as an experimental control instead of a standard mock community of 10 to 20 species of bacteria. Jacobs also questions the research implications of using The BioCollective's reference: "I'm not aware of data demonstrating that this [reference standard] has a significant effect in actual studies," he says.

In addition to working with NIGMS, Carlin is now collaborating with NIST, the International Life Sciences Institute, Caltech microbiologist Sarkis Mazmanian, and numerous other researchers, although her partnership with Gilbert has ended for now. "We're looking at how systems work together," she says, "and how people work together," adding that the scientific community could benefit from more openness and information sharing.

Nearly 18 years after receiving his diagnosis, John is boxing, biking, and leading Parkinson's support groups. He no longer needs the support of a cane to walk, and his scores in the Uniform Parkinson's Disease Rating Scale, which measures motor and non-motor symptoms, have improved over the last three years.

Carlin is determined to keep moving forward, just as she is dedicated to John and their shared belief that his disease is not a death sentence. The answer lies in the gut, she's convinced, and she'll keep searching until she finds it.

—Amy Schleunes

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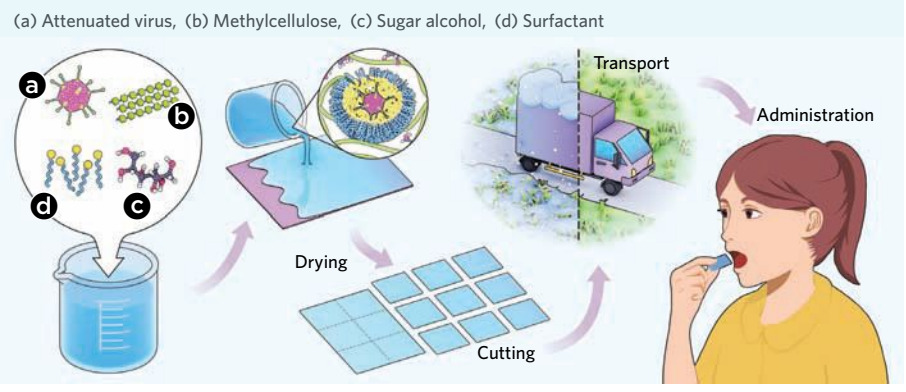
# Vaccines on Film

A novel preparation technique could facilitate vaccine preservation, transportation, and administration.

BY RUTH WILLIAMS




**NO VIALS, NO FRIDGES, NO NEEDLES:** To make a film-based vaccine, a mixture containing attenuated virus, methylcellulose, sugar alcohol, and surfactant would be poured out as thin layers and allowed to air dry in sterile conditions for eight hours. These flexible, lightweight sheets could then, in principle, be packaged and transported without the need for vials or protection from extreme temperatures. At their destination, the vaccines would be given orally to subjects, thus eliminating the need for syringes, needles, and trained personnel.

Vaccinations have revolutionized public health in the developed world, slashing the numbers of infectious disease–related deaths dramatically. But vaccine availability is often limited in low-income countries with poor healthcare infrastructures, leading to avoidable deaths. Two issues influencing the accessibility of many vaccines are their needs for constant refrigeration from production until use and for trained personnel to administer injections.

To overcome the issue of temperature sensitivity, some vaccines are freeze-dried, a process known as lyophilization, and transported as powders. But, says Maria Croyle, a pharmacologist at the University of Texas at Austin, in some instances the stability of lyophilized vaccines is less than ideal. For instance, some can be damaged if they freeze during transport or storage. Moreover, lyophilized vaccines still need to be reconstituted and injected by professionals.

To avoid these barriers, Croyle and her team have developed a vaccine preparation technique that both imparts temperature stability and allows easy administration. The vaccine—which for testing purposes is a live virus—is first added to a thick mixture of methylcellulose, sugar alcohol, and a surfactant. Then, under sterile conditions, the mixture is poured into thin layers and air-dried. "It's like how you would make taffy," Croyle says. The key to the virus's survival, says Croyle, is the molecules of surfactant, which form "a bubble around each virus particle," suspending and protecting it.

The result is a thin, peelable film containing live virus that can be stored at room temperature for up to three years and can maintain viability through repeated cycles of freezing and thawing. Subjects would then take the vaccine by allowing a small square of it to dissolve in their cheek or under the tongue.

In proof-of-principle experiments, Croyle's team prepared H1N1 influenza virus as a film and showed that its administration to the oral mucosa of mice induced antibody titers comparable to those obtained with standard intramuscular injection.

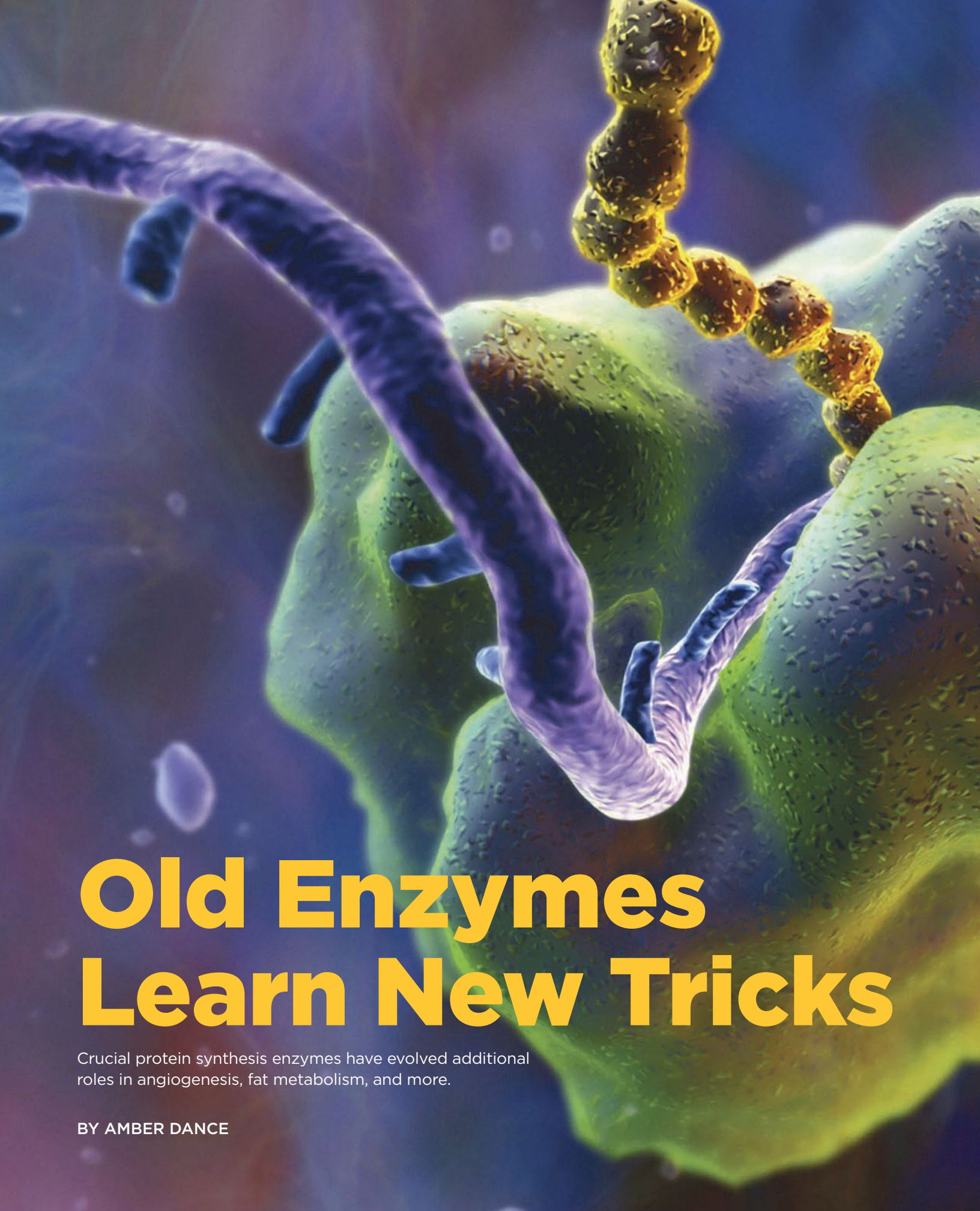
"Transportation of vaccines, particularly into the developing world, has always been a major issue, and this would seem to . . . overcome most of the known problems," says Russ Middaugh, a pharmaceutical chemist at the University of Kansas who was not involved in the work.

"It's a very important paper and extremely timely . . . in light of the mad rush to come up with [and distribute] a vaccine for COVID-19," adds pharmaceutical scientist Kishor Wasan of the University of Saskatchewan and University of British Columbia who also did not participate in the research. "This is huge," he says.

Croyle now plans to automate the film preparation process to enable production at the necessary scale for a vaccine. (*Sci Adv*, 6:eaau4819, 2020) ■

## AT A GLANCE

| VACCINE PREPARATION | HOW IT WORKS | TEMPERATURE STABILITY | ADMINISTRATION | CLINICAL USE |
|---|---|---|---|---|
| Lyophilization | A vaccine solution is freeze-dried into a powder for shipping and storage. | Good, but some lyophilized vaccines are damaged by subsequent freezing | Vaccine is reconstituted in liquid and then, in most cases, injected. | Well established, with approximately a dozen vaccines prepared in this way |
| Film | A virus is mixed with methylcellulose, sugar alcohol, and surfactant and poured into thin sheets, which air-dry at room temperature. | Viability of live virus remains close to 100 percent after repeated freeze-thaw cycles. Viability at 37 °C also largely unchanged after 15 days | A single-dose film is placed on the mucosa of the cheek or under the tongue. | So far, mouse experiments only |



# Old Enzymes Learn New Tricks

Crucial protein synthesis enzymes have evolved additional roles in angiogenesis, fat metabolism, and more.

BY AMBER DANCE

For as long as living things have been building proteins based on the code carried by messenger RNA molecules, aminoacyl-tRNA synthetases have been there. These enzymes, AARSs for short, link transfer RNAs (tRNAs) to the corresponding amino acids. That would seem to be a big enough job for one class of enzymes—and when protein-based life began, it was. But as organisms became more complex, AARSs picked up additional domains that allow them to do much more.

"By the time you get to humans, the synthetase has become highly decorated" with those additional domains, says Paul Schimmel, a Scripps Research Institute biochemist who studies these add-on jobs.

Living things possess at least one type of AARS molecule for each of the 20 proteinogenic amino acids. For some amino acids, there are two varieties, with a separate enzyme for use in protein translation that takes place in the mitochondrion. All of these synthetases have a core segment that is involved in linking tRNAs and amino acids, and all but one harbor one or more additional accessory domains. Plus, by alternatively splicing their mRNAs or fragmenting the proteins post-translationally, cells can make more than 300 different protein variants from AARS genes. Some of these variants moonlight as inflammatory cytokines. Others orchestrate the formation of blood vessels. The AARSs for glutamic acid and proline are merged into a two-part protein; the linker between them seems to control immune activity and fat metabolism, and may even influence life span. Many AARSs have been linked to human diseases caused by defects not in protein assembly, but in these other, noncanonical functions.

Some researchers now view the enzymes as drug targets for cancer, immune disease, and other conditions. The company Schimmel cofounded, aTyr Pharma in San Diego, envisions the AARS proteins themselves as an entirely new class of drugs, distinct from small molecules or other biologics. The firm is currently running a clinical trial testing a piece of the histidine enzyme, HisRS, for treating inflammatory lung disease.

Alternative AARS functions have been known in lower organisms such as bacteria since the 1980s, but their activities aren't extensive, says Schimmel. Then, starting in the '90s, Schimmel and others began to uncover noncanonical functions in higher eukaryotes, starting with unexpected roles in angiogenesis. The discovery of new functions for these ancient proteins was "a big surprise," says David Dignam, a biochemist at the University of Toledo. But given the diverse functions that researchers studying AARSs have uncovered, many of which touch on crucial disease pathways, Dignam says he thinks aTyr's approach makes sense. "Arguing that you can make medicines based on this, I think, is very logical."

While other proteins have adopted secondary functions, the quantity and variety of side gigs found in the AARSs is remarkable, says Schimmel. And he doesn't think it's a coincidence. These particular synthetases have been present and available for evolution to modify since protein-based life began. Given their essential role in protein synthesis, they're consistently produced, and unlikely to disappear from any viable genome. That makes them a stable substrate for new functional domains. Moreover, they possess specific amino acid binding sites, ready to interact with other proteins.

"It's lock and key," says Schimmel. "Any protein that sticks out a nice side chain that matches a synthetase could eventually become a partner."

## Building and blocking blood vessels

Schimmel says he's long been fascinated with AARSs' original function: interpreting the genetic code. Back in the '90s, Schimmel's lab, then at MIT, was sequencing the AARS genes. "We were interested in developing small molecules that would target them and kill their activities in specific ways," he says. For example, if the AARS of a pathogen was different enough from that in people, he reasoned, one could develop an antibiotic that shuts off protein synthesis in the infectious agent.

Schimmel's then-postdoc Keisuke Wakasugi got curious about the sequence of the gene encoding TyrRS, the AARS for tyrosine. In humans, TyrRS includes an extra segment at the carboxyl end of the enzyme, a feature that isn't present in prokaryotes or lower eukaryotes. The amino acid sequence for this part of the protein was similar to that for a human cytokine, EMAP II, which recruits circulating immune cells into tissues to promote inflammation. Wakasugi

decided to test that carboxyl domain for cytokine-like activity.

"That's a silly idea," Schimmel recalls thinking. But Wakasugi went ahead, and sure enough, the TyrRS carboxyl domain acted just like EMAP II, inducing cultured phagocytes and leukocytes to migrate and release inflammatory signals. The full-length TyrRS, in contrast, didn't influence the cells' behavior. That hinted at the possibility that the carboxyl domain could be broken off the TyrRS for immune functions. No one in the lab would believe the finding at first, so Wakasugi repeated the experiments, with the same results.

Although it would take more than a decade to show that such AARS fragments were truly present and relevant in a living animal, Wakasugi knew he was onto something. "Paul and I were very excited to discover a novel and unexpected function of human TyrRS," recalls Wakasugi, now a biochemist at the University of Tokyo. "Throughout this project, I felt that we opened the door to a whole new research field."

As part of the same study, Wakasugi also investigated the amino-terminal, catalytic domain of TyrRS, wondering if it might also influence cell migration. It behaved in a manner reminiscent of the cytokine interleukin-8 (IL-8). Both the TyrRS amino-terminal fragment and IL-8 bind to the IL-8 receptor on certain leukocytes, causing them to migrate in culture.[1]

Schimmel recruited Xiang-Lei Yang, a postdoc with expertise in structural biology, to join his lab at Scripps in La Jolla, California, to investigate how TyrRS might manage alternative functions. Yang zeroed in on a particular sequence of amino acids, glutamic acid–leucine–arginine, required for the synthetase fragment's cytokine activity. The same sequence was also found in IL-8 and related cytokines. In crystal structures, she found that full-length TyrRS buried this motif, but it was exposed in the cytokine-like fragment.[2]

IL-8 was known to promote the formation and growth of blood vessels, so Wakasugi also tested his TyrRS amino-terminal fragment for angiogenic activity. When he injected a bit of gel containing the fragment into mice, blood vessels grew and suffused the gel.[3] To explore that action further, Schimmel phoned his Scripps colleague Martin Friedlander, an ophthalmologist and cell and developmental biologist, and asked him to test the TyrRS fragment in his mouse models of eye vascularization. Friedlander agreed, but also asked for a control. So along with the human TyrRS fragment, Wakasugi provided a natural splice variant of the tryptophan enzyme, TrpRS, that lacks the glutamic acid–leucine–arginine motif.

The results, Friedlander recalls, weren't exactly what he expected. TrpRS, the supposed control, "had a much more potent effect," says Friedlander, who is also president of the Lowy Medical Research Institute in La Jolla. But that effect was the opposite of TyrRS action: rather than promote angiogenesis, as Wakasugi had seen in the gel, the TrpRS fragment blocked it in mammalian cell culture, chicken embryos, and mouse eyes.[4,5] "TyrRS and TrpRS may have evolved as opposing regulators of angiogenesis," says Wakasugi.

Scientists were initially resistant to the idea that an AARS could have functions beyond protein synthesis. Yang recalls attending a conference, shortly after Wakasugi published his work on angiogenesis, where others were unaware that she was a Schimmel acolyte. Thus incognito, "I heard how skeptical the field was about those discoveries," she recalls. "I don't blame them. I would be confused too."

## Vasculature and beyond

While the TyrRS and TrpRS functions Wakasugi and colleagues had discovered were interesting, it wasn't clear that the enzyme fragments genuinely performed these functions in vivo. Yang realized that to give herself and other scientists confidence about noncanonical functions of AARSs, she'd have to find evidence that they were present in animals.

The team still hasn't done so for TrpRS or TyrRS, but Wakasugi found her opportunity with the serine enzyme, SerRS. Multiple published genetic screens in zebrafish had identified defects in vascular development when SerRS was mutated. But mutations that knocked out the enzyme's ability to link tRNAs and amino acids did not cause such defects, indicating that something else was going on.

**I heard how skeptical the field was about those discoveries. I don't blame them. I would be confused too.**

—Xiang-Lei Yang, Scripps Research Institute

To figure out what, Yang turned to a sequence, christened UNE-S, that is found in vertebrate, but not invertebrate, SerRS. Yang's team—she joined the Scripps faculty in 2005, and now shares a lab with Schimmel—quickly identified a nuclear localization sequence within UNE-S, and determined that mutations altering this signal caused the vascular defects in zebrafish embryos. In the nucleus, they found, SerRS seems to minimize the expression of vascular endothelial growth factor A (VEGFA).[6] The study, published in 2012, was the first to illustrate an essential, natural role for an AARS accessory domain in a living animal. Shortly thereafter, the team reported that nuclear SerRS blocks *VEGFA* by competing and interfering with c-Myc, a transcription factor that normally promotes the gene's expression.[7]

Meanwhile, Schimmel's and Yang's groups continued to try to explain the noncanonical functions of TrpRS and TyrRS, even as they found more side gigs for these enzymes. Yang led studies on the TrpRS fragment's structure and mechanism. She discovered that full-length TrpRS doesn't influence angiogenesis because it's capped by a WHEP domain[8]—so called because this domain appears in aminoacyl tRNA synthetases for tryptophan (W), histidine (H), glutamic acid (E), and proline (P), as well as in the glycine and methionine

enzymes. Yang's team found that when uncapped by proteases in the extracellular space, TrpRS binds to a cellular receptor called VE-cadherin. Specifically, tryptophans in the receptor seemed to enter the TrpRS's active site to create the bond.[9] That's why Wakasugi saw that only the fragment, not the full TrpRS, blocked angiogenesis.

More recently, Schimmel has also been interested in plant-based amino acid–like compounds, such as resveratrol, the stuff in red wine that's thought to counter oxidative stress. Resveratrol and tyrosine are similar in that both contain a phenolic ring, and that's important for resveratrol's ability to influence the expression of pro- and anti-oxidative genes. In 2015, Schimmel's team reported that under conditions of stress, TyrRS moves into the nucleus of human cultured cells or living mice, where any resveratrol present fits neatly into TyrRS's active site. This turns off the normal TyrRS catalytic activity to connect tyrosine molecules with the appropriate tRNAs. Instead, TyrRS stimulates the activation of PARP-1, an enzyme involved in DNA repair.[10]

A few years later, the team found that an alternatively spliced version of TyrRS stimulates platelet proliferation in mice and cultured cells, and could potentially be used to treat people with a low platelet count.[11]

Schimmel expects noncanonical AARS functions will keep the group busy for a long time. "We are barely scratching the surface of what is to be learned," he says. "I am as excited, or even more excited, about these enzymes as I was when I started out decades ago."

## Managing inflammation and metabolism

As evidence of noncanonical functions for AARSs was trickling out of Schimmel's lab, Paul Fox, a biochemist at the Cleveland Clinic's Lerner Research Institute, was studying the control of inflammation in macrophages. Specifically, his team was investigating a complex generated when the cells were exposed to the cytokine interferon-γ. A protein complex called GAIT (for interferon-γ activated

### THE DIVERSE FUNCTIONS OF SYNTHETASES

Aminoacyl tRNA synthetases are crucial players in protein synthesis, linking tRNAs to the amino acids dictated by the codon sequence. All AARSs have also been found, in diverse in vitro and in vivo systems, to play non-protein synthesis roles in a number of body systems. This table includes a sampling of the more well-studied examples.

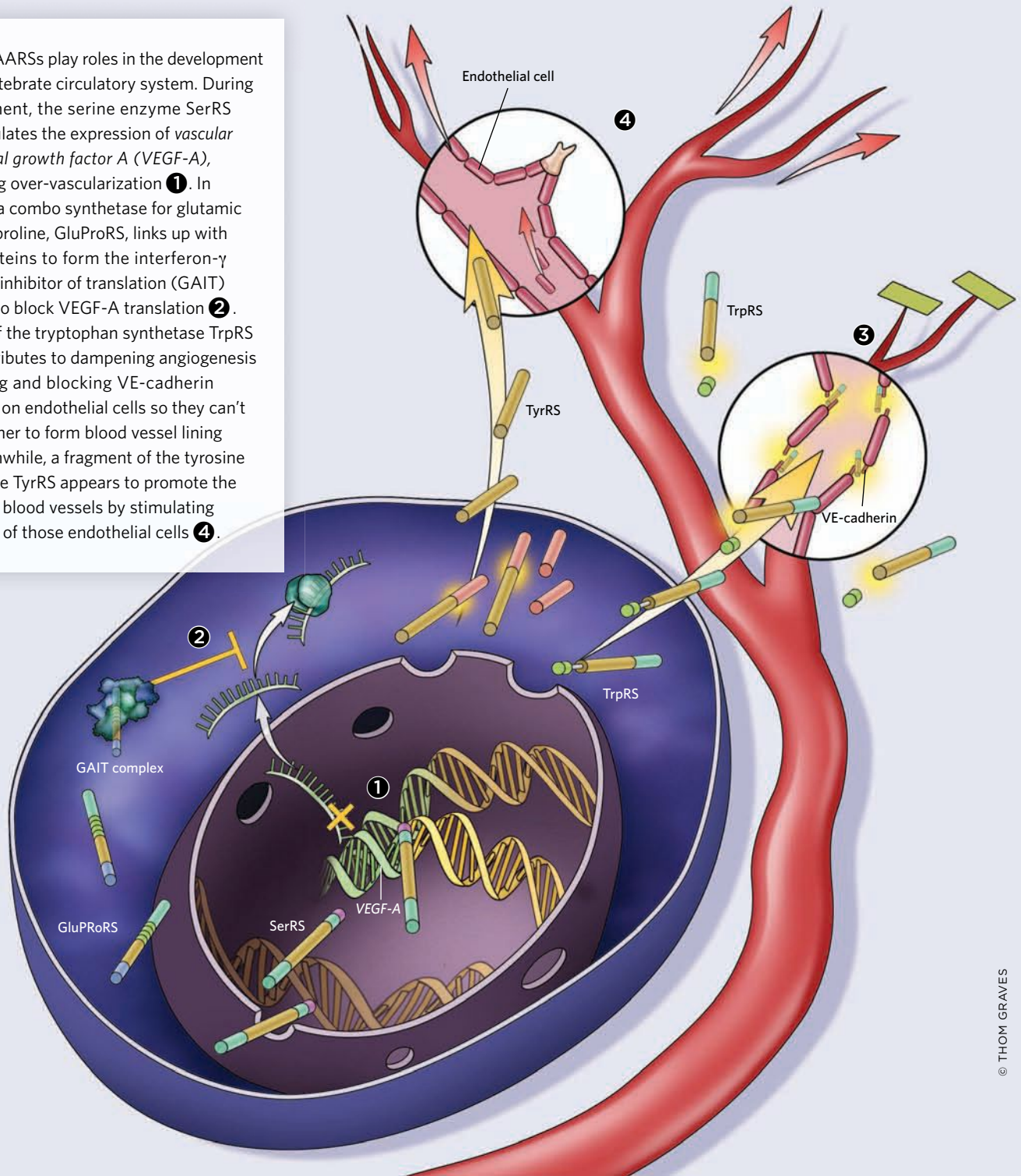
| | |
|---|---|
| METABOLISM | • **LeuRS**, the synthetase for leucine, participates in a pathway to sense cellular levels of the amino acid leucine. |
| VASCULATURE & ANGIOGENESIS | • A fragment of the tyrosine synthetase, **TyrRS**, promotes angiogenesis.<br>• **TrpRS**, the synthetase for tryptophan, restricts angiogenesis by blocking a molecule that endothelial cells use to link together and build blood vessels.<br>• **SerRS**, the synthetase for serine, downregulates the expression of *VEGF-A*, preventing over-vascularization during development.<br>• **GluProRS**, a single protein that includes the AARSs for glutamic acid and proline, influences vasculature by blocking the translation of *VEGF-A*. |
| CELL CYCLE & TUMORIGENESIS | • **GluProRS**'s actions on *VEGF-A* also promote tumor growth.<br>• **GlnRS**, the synthetase for glutamine, blocks the proapoptotic pathway of an enzyme that controls tumorigenesis and stress responses.<br>• **TrpRS** bridges two nuclear proteins to activate the production of the cell cycle regulator and tumor suppressor p53. |
| IMMUNITY, INFLAMMATION, & INFECTION | • **LysRS**, the synthetase for lysine, assists in the synthesis of a molecule that activates the transcription of genes involved in immune regulation.<br>• During HIV infection, **LysRS** is packaged into new viral particles that use its UUU sequence to prime reverse transcription in newly infected cells.<br>• **GluProRS** suppresses the translation of mRNAs involved in inflammation.<br>• **TyrRS** fragments act as inflammatory cytokines. |

*Nat Chem Biol*, 9:145–53, 2013 and *FEBS Letters*, 584:434–42, 2010

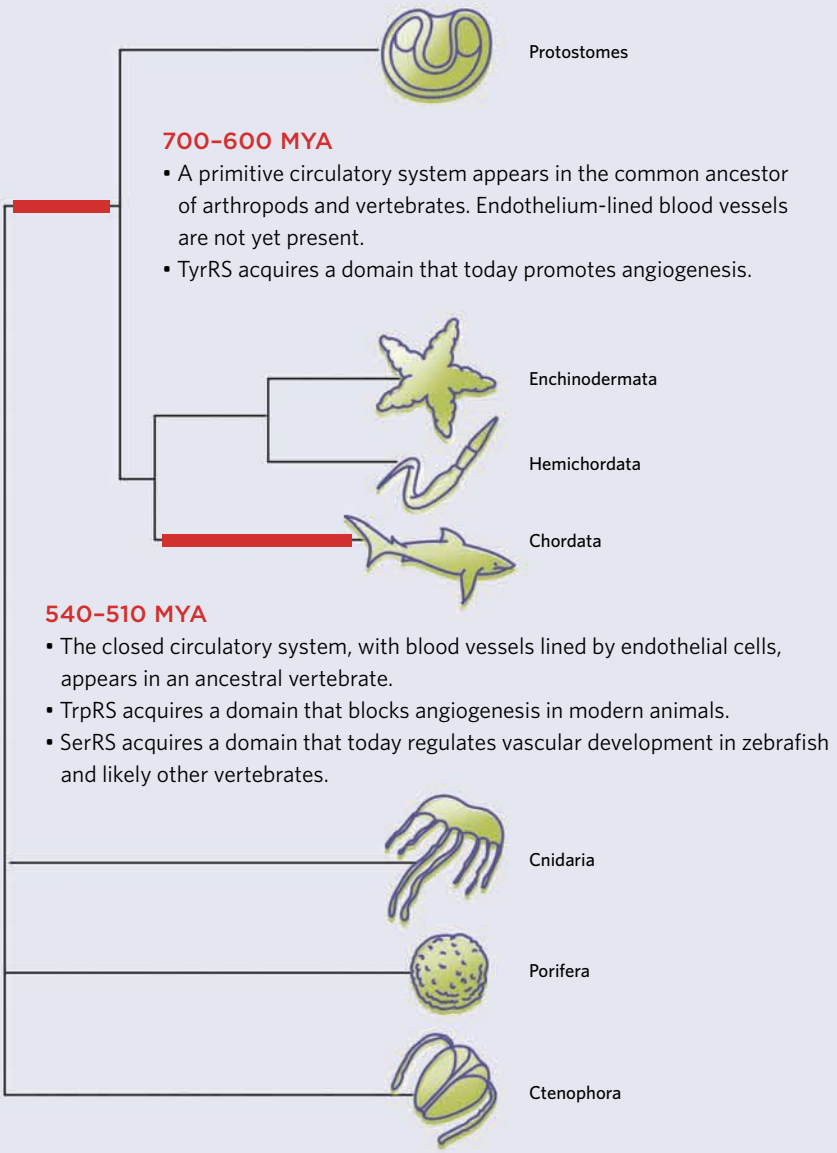
# MOONLIGHTING ENZYMES

Aminoacyl-tRNA synthetases play a fundamental role in protein translation, linking transfer RNAs to their cognate amino acids. But in the hundreds of millions of years that they've existed, these synthetases (AARSs) have picked up several side jobs. One of these is to manage the development of vertebrate vasculature.

Multiple AARSs play roles in the development of the vertebrate circulatory system. During development, the serine enzyme SerRS downregulates the expression of *vascular endothelial growth factor A (VEGF-A)*, preventing over-vascularization ❶. In addition, a combo synthetase for glutamic acid and proline, GluProRS, links up with other proteins to form the interferon-γ activated inhibitor of translation (GAIT) complex to block VEGF-A translation ❷. A piece of the tryptophan synthetase TrpRS also contributes to dampening angiogenesis by binding and blocking VE-cadherin receptors on endothelial cells so they can't link together to form blood vessel lining ❸. Meanwhile, a fragment of the tyrosine synthetase TyrRS appears to promote the growth of blood vessels by stimulating migration of those endothelial cells ❹.



## WHEN THESE FUNCTIONS AROSE IN EVOLUTION




According to Scripps Research Institute biochemist Paul Schimmel, the addition of accessory domains that perform such tasks parallels major events in the evolution of circulation. The first blood vascular system, which lacked the endothelium present in modern vertebrates, probably arose in a common ancestor of vertebrates and arthropods around 700 million to 600 million years ago. Around this same time, TyrRS acquired a glutamic acid–lysine–arginine motif that today is thought to promote angiogenesis. Then, around 540 million to 510 million years ago, an ancestral vertebrate evolved a closed vascular system, with blood pumping through vessels lined by endothelium. At some point around that same time period half a billion years ago, the TrpRS picked up a WHEP domain, which today regulates its ability to block angiogenesis. In addition, SerRS acquired a domain unique to this enzyme, which now prevents over-vascularization in developing zebrafish, and likely other vertebrates.

GluProRS's role in angiogenesis, on the other hand, doesn't seem to be so precisely timed to the evolution of vasculature. A linker protein tied together the AARSs for glutamic acid and proline enzymes around 800 million years ago, before circulatory systems existed.

inhibitor of translation), generated within macrophages, binds to and blocks mRNAs related to inflammation. Inside the complex, the researchers found GluProRS, an enzyme that includes the AARSs for glutamic acid and proline.

"We ran into it just absolutely by accident," Fox recalls. "I didn't think it was an interesting enzyme." But he knew of Schimmel's work, and he picked up the phone to call Scripps.

One minute into the call, Schimmel interrupted to welcome Fox to what Schimmel called the most exciting area of AARS research: the noncanonical functions. Schimmel also promised his assistance, Fox says. "He's been a big supporter and a friend ever since." With tools supplied by Sunghoon Kim, a former Schimmel lab postdoc now at Yonsei University in South Korea, Fox's team discovered that interferon-γ causes GluProRS to become phosphorylated, abandon its post in translation, and join up with the other GAIT members to halt the production of inflammatory proteins.[12]

It's not clear why the glutamic acid and proline synthetases paired up approximately 800 million years ago, but Fox has a hypothesis, which he published in 2018.[13] Proline is synthesized from glutamic acid, and at that period in evolution, emerging animal proteins began to include more proline. That may have led to a rise in the production of ProRS that sopped up all the available proline, requiring more to be made from glutamic acid. That might have resulted in a deficit in glutamic acid levels, impairing protein synthesis. "The solution to that was to fuse the two synthetases into a single gene, so they have to be made in the same amounts," says Fox. "No one's stealing from the other."

The linker between the two synthetases is crucial for GAIT complex activity; it's made of three WHEP domains that bind to target RNAs.[14] Fox speculates that sometime after the linker appeared, the cell coopted it to regulate inflammation.

More recently, Fox's team wondered if the GAIT pathway might function in cells besides macrophages. When the researchers looked at fat cells, they saw that insulin treatment caused GluProRS to become

phosphorylated and leave the protein-synthesis machinery. But it didn't join the other GAIT partners. Instead, it paired with a normally cytosolic protein called fatty acid transport protein 1 (FATP1). Together, the molecular duo went to the fat cell's membrane, where the transporter brought fatty acids into the cell.

The researchers engineered a mouse lacking the phosphorylation site needed to free GluProRS to find FATP1. With less fatty acid–storage ability, the mice were lean, weighing about 15 percent to 20 percent less than control animals. Moreover, they lived nearly four months longer, giving them a lifespan that was increased by about 15 percent.[15] A similar gain in people would correspond to a decade or more. "If we could target that phosphorylation site, maybe we could increase lifespan," says Fox. His lab is in the very early stages of looking for a small molecule to inhibit that phosphorylation event.

**I am as excited, or even more excited, about these enzymes as I was when I started out decades ago.**

—Paul Schimmel, Scripps Research Institute

## Drug development

In the various jobs that AARSs have taken on above and beyond their traditional role, Schimmel and colleagues see a theme: they keep cells and bodies stable. "They seem to be something that's playing a modulatory role, restoring more of a homeostasis," says Leslie Nangle, a former Schimmel lab grad student who is now senior director for research at aTyr Pharma. Many researchers think it's risky to mess with such essential enzymes, says Kim, but he and Schimmel see potential in targeting AARSs for treating disease. Schimmel's company aTyr, of which Kim and Yang are also cofounders, hopes to turn the enzymes themselves into biologic therapeutics. In addition, in Seoul, Kim directs the nonprofit drug discovery organization Biocon, where researchers are developing several small molecules that interact with AARSs, as well as biologics based on natural AARS variants.

Biocon is currently testing molecules to treat cardiac fibrosis, alopecia areata (an autoimmune disease that causes hair loss), and inflammation. A fibrosis treatment now under Phase 1 study targets the site on the proline synthetase that links the amino acid to its tRNA. Fibrosis results from an accumulation of collagen, which is two-thirds proline. Biocon researchers have found that a drug can go after that active site, knocking down the canonical function by more than 90 percent in healthy cultured cells without greatly affecting the synthesis of other proteins or cell proliferation, says Kim. At first, he and his colleagues didn't believe their results, but he's come to see sense in them. "A normal cell is not necessarily doing high level protein synthesis all the time," he says. "As long as it has a certain degree of residual activity going on, then a normal cell can be perfectly happy."

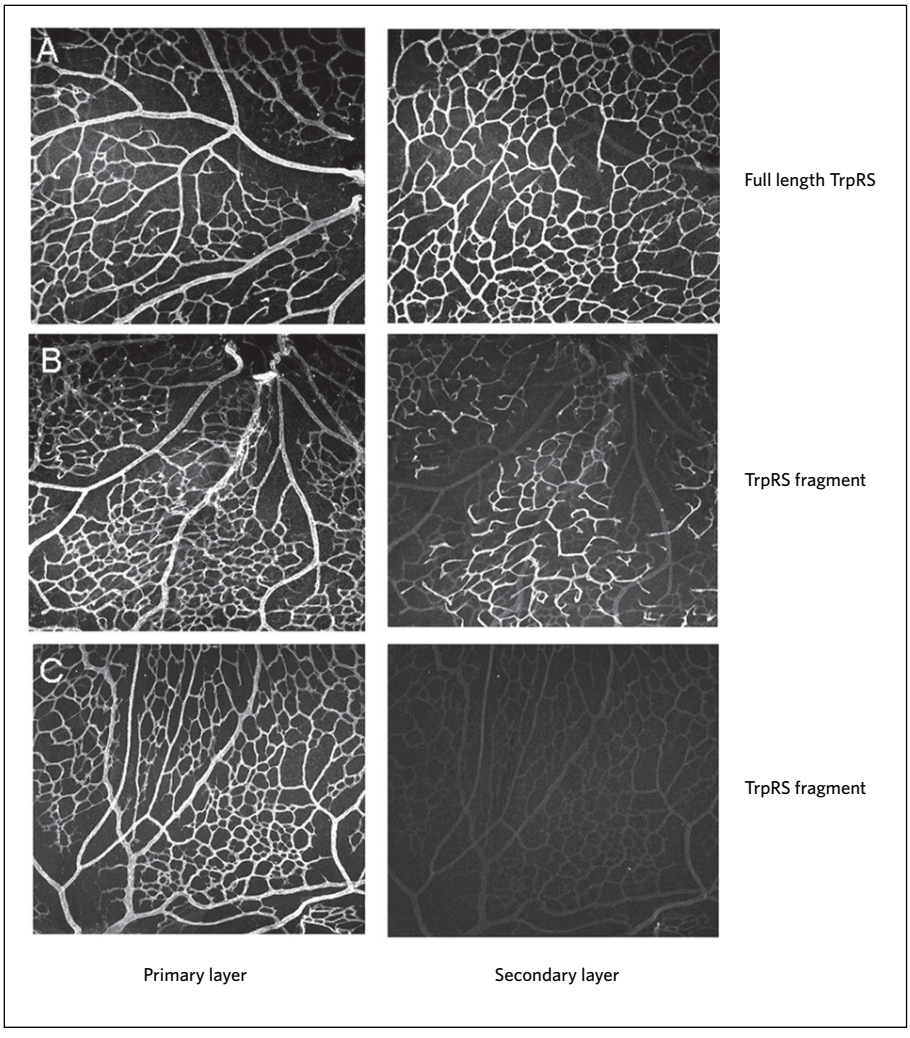
For cancer and other conditions, Biocon is developing small molecule candidates that avoid the tRNA–amino acid linking site or target the extracellular activities of secreted AARSs, meaning that protein synthesis should not be affected. Similarly, aTyr researchers expect that the firm's therapeutics, based on AARS derivatives themselves, to be relatively safe. "Coming from a world of natural physiology, you start to feel better about it," says aTyr CEO Sanjay Shukla.

Nangle and colleagues, alongside aTyr's subsidiary Pangu Biopharma in Hong Kong, began by cataloging natural AARS splice variants and then screening them for interesting biological activities in a variety of human cell–based assays. They looked for effects on cell proliferation and protection, immunomodulation and inflammation, cell differentiation, transcriptional regulation, and cholesterol transport.[16] "We figured there's got to be some therapeutic benefit there," says Schimmel.

Currently, aTyr is pursuing an immunomodulator based on the WHEP domain of the histidine enzyme HisRS. In human T cell cultures, full-length HisRS quieted activated cells and reduced cytokine production.[17] In further experiments, aTyr researchers found that the WHEP domain hooks up with receptors on those immune cells to dampen activity. The company hopes that its modified version of the HisRS WHEP peptide, attached to a bit of antibody to help it last longer in the bloodstream, will have the same quieting effect in an inflammatory disease called sarcoidosis. This disease affects a variety of organs, most often the lungs, and can sometimes require lifelong treatment with immune-suppressing steroids. Those medications come with a list of misery-inducing and dangerous side effects ranging from insomnia to glaucoma to infection.

aTyr presented results from several animal models of inflammatory lung disease at the American Thoracic Society meeting in 2017, 2018, and 2019, and those findings suggest the company's candidate 1923 looks promising. For example, the cancer drug bleomycin can cause lung damage, but HisRS or its WHEP domain reduced inflammation and fibrosis.[19] Rats treated with bleomycin breathe quickly to compensate for their damaged lungs, but those treated with 1923 recovered normal respiratory rates.

aTyr's 1923 has already been through a Phase 1 trial for safety in healthy people without any red flags. Now, the company is running a Phase 1/2 study in people with sarcoidosis, looking to confirm safety, find the right dosage, and perhaps even see signs of efficacy. Patients enter the trial while taking steroids, and the aim is to taper down the steroid dosage during the study. Those receiving 1923, it's hoped, will see their symptoms stay the same or improve, while those on placebo should see them worsen as the steroid doses are lowered.




VASCULATURE DENIED: In the developing mouse retina, fragments of the tryptophan synthetase, TrpRS, that are missing a restrictive protein cap (B, C) prevent vascularization of a secondary tissue layer (right). (The right images include the shadows of the adjoining primary layers, which are shown at left.)

It's a testament to the need for a new treatment that volunteers are willing to risk having their symptoms intensify if they end up in the placebo arm, says participating physician Daniel Culver, a pulmonologist at the Cleveland Clinic. "[Steroids are] very toxic," says Culver, who notes that one of his patients calls his steroid prescription "the Devil's drug" because it does almost as much harm as good. "People are very, very motivated to find something different."

The study plans to enroll 36 participants, but has been delayed by the COVID-19 crisis. With such a small sample size, Culver doesn't expect a "home run," but he says he hopes the data will be good enough to embark on a larger, Phase 3 study. aTyr is also planning a Phase 2, 30-person trial of 1923 for respiratory complications associated with COVID-19.

If aTyr succeeds, it will be the first instance of a therapeutic built from an AARS—but probably not the last. As Kim sees it, AARSs are ready and waiting to respond to anything that challenges homeostasis, from cancer to the novel coronavirus. "I rename the synthetases 'Molecular 911.'" ■

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# DNA's Secret Code

Once considered an unimportant curiosity, left-handed DNA is now recognized as part of a dynamic genetic system that regulates how an RNA transcript is edited.

BY RACHEL BRAZIL

In 1970, biochemist Robert Wells of the University of Alabama at Birmingham saw something strange in his X-ray images of a new synthetic DNA polymer. The DNA molecule was composed of the traditional sugar backbones and nucleotide pairs, but rather than the well-known right-handed spiral of the double helix structure, famously discovered by Watson and Crick in 1953, Wells's polymer spiraled in the opposite direction, giving it a zigzag appearance.[1]

Whether this bizarre form of DNA existed in cells and had any function, and what that might be, was hotly debated for nearly half a century. But research has recently confirmed its biological relevance. So-called Z-DNA is now thought to play roles in cancer and autoimmune diseases, and last year scientists confirmed its link to three inherited neurological disorders. Today, molecular biologists are beginning to understand that certain stretches of DNA can flip from the right- to the left-handed conformation as part of a dynamic code that controls how some RNA transcripts are edited. The hunt is now on to discover drugs that could target Z-DNA and the proteins that bind to it, in order to manipulate the expression of local genes.

The story of Z-DNA is an unusual one, says Alekos Athanasiadis, an expert on protein–nucleic acid interactions at the Gulbenkian Science Institute in Portugal. "Usually you have a biological function and then structural work is used to identify the mechanism behind it," he says. "In this case, the research community was starting from structural information and the biology followed."

## Looking for a biological function

In 1979, working as postdocs in the laboratory of the late Alexander Rich at MIT, Andrew Wang and Gary Quigley solved the left-handed DNA structure that Wells had observed several years earlier.[2] In contrast to right-handed B-DNA, which has

two differently sized gaps known as the major and minor grooves between the twists of its sugar-phosphate backbone, the left-handed form, which Rich dubbed Z-DNA, has grooves that are much more uniform. In addition, every other base takes a slightly different orientation compared to how it sits in B-DNA, giving the helix its zigzag structure. (See illustration on opposite page.)

B-DNA and Z-DNA typically coexist on a double-stranded DNA molecule, with stretches of anywhere from a dozen to 100 base pairs taking on the reverse spiral structure. The point in the nucleic acid where the direction of its spiral changes is known as a B-Z junction.[3] Z-DNA is quite transient, quickly flipping back to the B conformation, sometimes within seconds. "[It] is a very dynamic process," says immunobiologist Alan Herbert, who worked for years as a researcher in Rich's lab at MIT before he founded a DNA-based therapeutics company called InsideOutBio in 2017. "You cycle between the Z and B." This makes it exceptionally challenging to study.

To try to understand whether Z-DNA has a biological function, Colorado State University structural biologist P. Shing Ho, another former member of the Rich group, and colleagues looked for potential Z-forming sequences in the human genome. Using Z-DNA specific antibodies to identify Z-DNA stretches in a variety of plasmids, the research team discovered that Z-DNA formed most readily in repeated sequences of alternating purines and pyrimidines, particularly the purine guanine (G) and the pyrimidine cytosine (C). Using an algorithm to identify more than 300 similar sequences across the genome, the team found that "the Z-DNA regions tend to cluster right around the transcription start sites of most eukaryotic genes," says Ho. "They are widely distributed across different types of genes, but they are not found within genes themselves."

Researchers have also found that Z-DNA formation is linked to the "supercoiling" of DNA molecules, which are twisted around themselves into tangled configurations. During transcription, as an RNA polymerase moves along the DNA strand, it causes over- and underwinding of the DNA, compressing or relaxing the double helix. "These changes produce strain on the molecular configuration in the vicinity of the enzyme," explains Burghardt Wittig, yet another former Rich lab member, now at the Free University of Berlin. The strain behind the polymerase changes the thermodynamic stability of the DNA molecule, making it more likely to flip to the Z-DNA conformation. In 1990, Wittig was able to detect Z-DNA in cells during active transcription, and showed that inhibiting RNA transcription decreased the amount of Z-DNA in the genome.[4]

> It's clear this has some important function in nature.
>
> —Burghardt Wittig, Free University of Berlin

This link to transcription led some researchers to speculate that Z-DNA provided some sort of epigenetic switch, turning gene transcription on and off. "But the data didn't really support that simplistic switch model," notes Herbert, and many scientists and funders gave up on the field altogether. But Herbert remained convinced that Z-DNA had a biological function, and was determined to prove it.

## Z-DNA's relevance revealed

Herbert took a big step toward convincing the field of Z-DNA's importance in 1995 when he and others at MIT discovered a Z-DNA binding protein.[5] The researchers found that the RNA-editing enzyme ADAR1 contained a domain, which they named Zα, that bound to Z-DNA. The Zα part of the protein binds to Z-DNA's backbone, rather than to any of the bases, and so is not specific to a DNA sequence, but to the left-handed conformation.

The Zα domain is present in a small number of other proteins in organisms from viruses to humans, but initially "it was not clear why these proteins bind to Z-DNA," says Wittig. The Zα domain seems to stabilize otherwise transient Z-DNA regions, so it's possible that the domain itself may induce B-DNA to flip to the Z formation,[6] rather than the Z-DNA attracting proteins with a Zα domain. "This is a chicken and egg question," says Chi-Hua Lee, a postdoc in the lab of Wang, Rich's former mentee, who is now at the Academia Sinica in Taiwan.

ADAR1 also binds to double-stranded RNA, which forms when an RNA transcript folds back and base pairs with itself. Normal cells produce a large number of double-stranded RNAs during routine transcription of genes. The ADAR1 enzyme is known to edit double-stranded RNAs—specifically, it helps change adenosine bases into inosine, a base which is read by ribosomes as guanosine when present in codons being translated. This base change hampers RNA's ability to take on a double-stranded conformation. Double-stranded RNA molecules initiate cell signaling pathways involving type 1 interferons, proteins that trigger immune responses that can damage cells. By editing double-stranded RNAs, ADAR1 limits the interferon immune response, ultimately stopping more-widespread cell damage.

One source of double-stranded RNA targeted by ADAR1 is Alu elements within the genome. These are transposable DNA stretches, also known as jumping genes, named for their identification using a restriction endonuclease extracted from *Arthrobacter luteus* (ALU) bacteria. Alu elements make up about 10 percent of the human genome and are known to produce "junk" double-stranded RNA in cells. Alu elements include many alternating purine-pyrimidine base sequences—which Herbert realized were the exact sequences that are known to form Z-DNA. So when the ADAR1 protein binds to these Z-DNA–forming regions, it is often close to an Alu element that yields double-stranded RNA that ADAR1 also acts on. Herbert suspected this was no coincidence.

Sure enough, Herbert's team last year published evidence of a causal link

between mutations in *ADAR1* that prevent the encoded protein from binding to Z-DNA and a number of inherited inflammatory diseases that involve the overproduction of type 1 interferons. By looking at multiple mutations in the human ADAR1 gene, Herbert showed that only those that led to changes in the Zα domain of the protein—not in the binding domains that recognize double-stranded RNA—were associated with the diseases, suggesting that it was the loss of the enzyme's ability to bind to Z-DNA that was causing the inflammatory symptoms.[7]

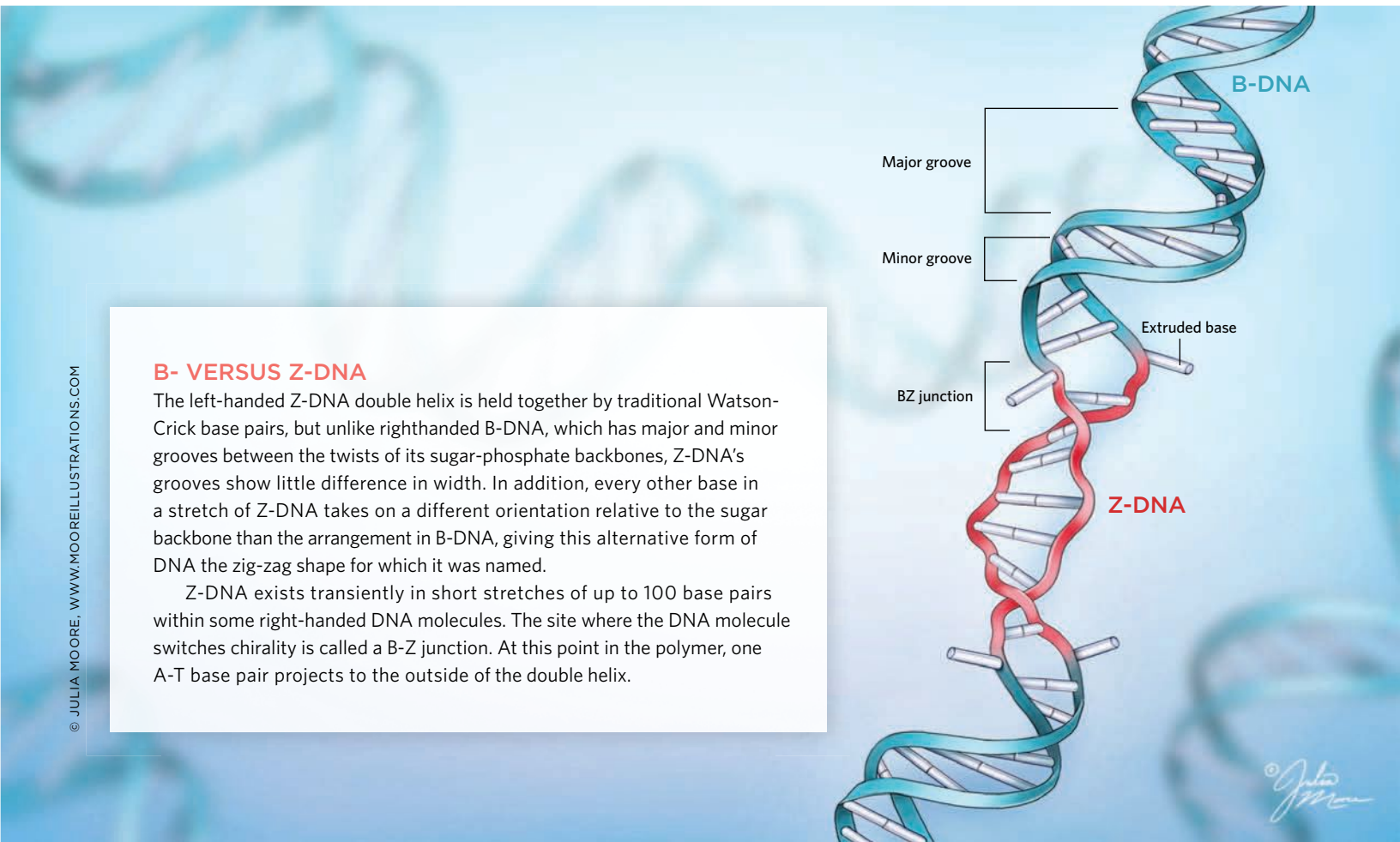
Herbert concluded that Z-DNA regions provide sites for ADAR1 to bind, allowing the protein to orchestrate the editing of double-stranded RNAs produced by transcription near the Z-DNA forming region. (See illustration on page 36.) "The Z-DNA domain is actually localizing the ADAR1 editing to those Alu elements within the genome, and this allows the RNA they produce to be edited, which in turn will then inhibit any interferon response," says Herbert. But patients with the rare diseases he studied have genetic mutations that prevent the enzyme's Zα domain from binding to these Z-DNA regions in the genome; "because [they're] not able to localize the enzyme where it needs to be, you are unable to take care of business and stop the interferon response from being amplified."

Wittig says that Z-DNA's link to these diseases was "the nail in the coffin" showing that the left-handed conformation of nucleic acids is biologically relevant. "It's clear this has some important function in nature," he says. "This is definitely the final proof." And these are likely not the only diseases in which Z-DNA plays some kind of role, Herbert adds. The involvement of immune response pathways also suggests that Z-DNA could be involved in other ailments, including cancer, and recent work points to Z-DNA or proteins that bind it as potential therapeutic targets. (See "Drugging the Z-conformation" on page 37.)

## A dynamic code

For Herbert, the biological relevance of Z-DNA is massive, as he suspects that flips in DNA chirality influence how RNA molecules are processed across the genome. He suggests that the formation of Z-DNA and the localization of Z-binding proteins during transcription could quickly turn on and off the editing of RNA products at many active genes.

Because Z-DNA is so unstable, Herbert named DNA sequences that can flip into the left-handed conformation "flipons." He hypothesizes that the final readout of genetic information from the genome depends on the activity of these flipons at the time of transcription. "It's not an on-and-off switch for the gene, but it does play a role in regulating how the initial transcript is compiled into different RNAs," he explains.




### B- VERSUS Z-DNA

The left-handed Z-DNA double helix is held together by traditional Watson-Crick base pairs, but unlike righthanded B-DNA, which has major and minor grooves between the twists of its sugar-phosphate backbones, Z-DNA's grooves show little difference in width. In addition, every other base in a stretch of Z-DNA takes on a different orientation relative to the sugar backbone than the arrangement in B-DNA, giving this alternative form of DNA the zig-zag shape for which it was named.

Z-DNA exists transiently in short stretches of up to 100 base pairs within some right-handed DNA molecules. The site where the DNA molecule switches chirality is called a B-Z junction. At this point in the polymer, one A-T base pair projects to the outside of the double helix.

Herbert suggests flipons take on the Z conformation only once transcription is underway, because the DNA supercoiling that accompanies active transcription is thought to promote the conformational change. But a 2012 study provided some evidence that Z-DNA may help open up the DNA that is normally tightly wound around histone proteins in nucleosomes, in preparation for transcription to begin. Keji Zhao of the National Heart, Lung, and Blood Institute found that a protein complex called SWI/SNF (SWItch/Sucrose Non-Fermentable), which is involved in loosening DNA-histone interactions, caused DNA near the promoter region of a gene to flip into the Z conformation.[8]

Zhao speculates that Z-DNA modulates the placement of nucleosomes on the genome. "Formation of Z-DNA by the activity of SWI/SNF complexes may first generate an unstable nucleosome, which can slide to a nearby B-DNA region or eject the core histones to form a nucleosome-free region," thus allowing transcription to start, he explains. The idea that Z-DNA could be present on DNA molecules wound around histones is somewhat surprising, notes Ho. "Most of the data that we've seen from other laboratories have shown that Z-DNA doesn't actually sit on nucleosomes, primarily because [Z-DNA] is a very stiff structure," he says. "It's very rod-like, whereas nucleosomes require a very large amount of flexibility in the DNA in order to make essentially 200 base pairs wrap around the small complex."

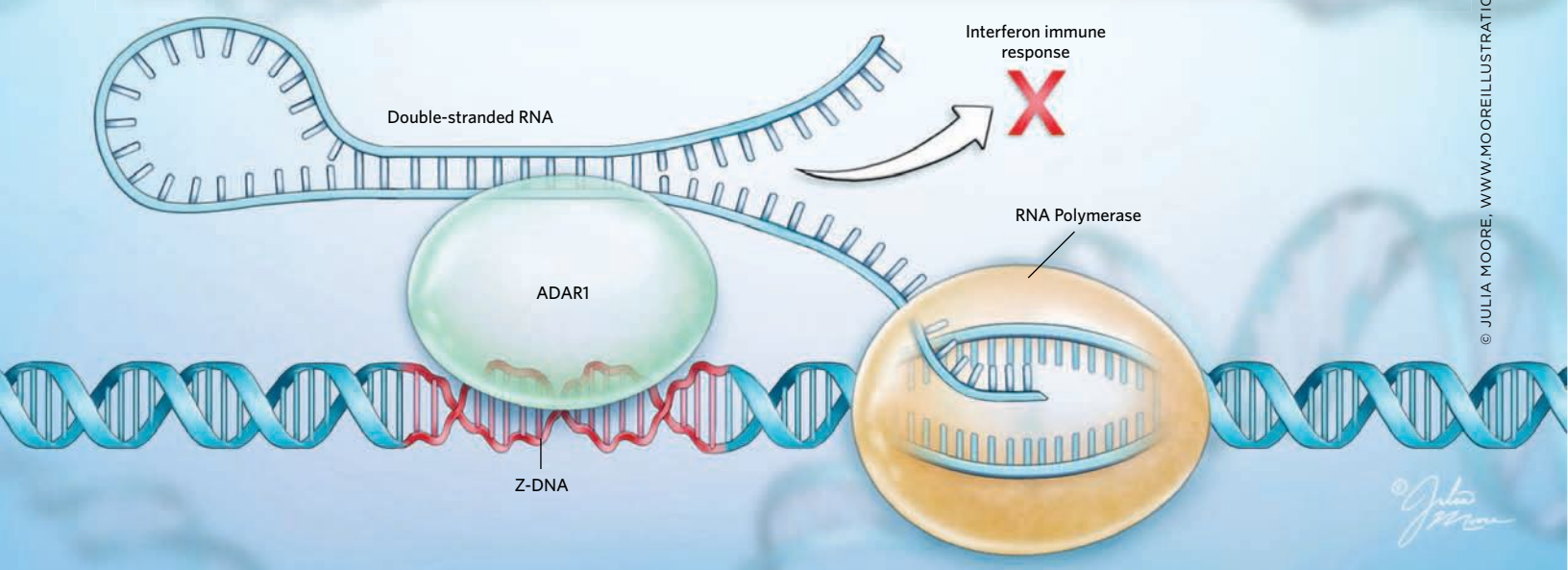
> It's a different way of thinking about the biology.
>
> —Alan Herbert, InsideOutBio

Zhao's work also supports the idea that Z-DNA formation may be influenced by DNA methylation. He and his colleagues created DNA templates assembled into nucleosomes that contained known Z-forming regions, including DNA with methylated and non-methylated guanine bases. The researchers could detect Z-DNA using a restriction enzyme modified with two copies of the Zα binding domain that would cleave the DNA if the Z configuration was present. The team found that Z-DNA was only present when the DNA fragments were made with methylated guanines. An older study had similarly found that DNA tends to switch conformations in the presence of methylated cytosines.[9] Herbert adds that there are other types of DNA modifications, such as the hydroxymethylation of cytosine, that make the formation of Z-DNA more difficult and favor the B-DNA conformation.

The link to epigenetics will need more investigation, but it has led Herbert to the idea that flipons ultimately have a quick and spontaneous role in controlling how cells react to

## Z-DNA IN ACTION

Z-DNA is linked to control of the interferon immune response through the RNA-editing enzyme ADAR1, which contains a Z-DNA binding domain called Zα. By editing double-stranded RNAs (dsRNAs), produced by stretches of repetitive DNA known as Alu elements, ADAR1 limits the interferon immune response normally caused by the dsRNA molecules. Recent work has shown that Z-DNA regions provide ADAR1 binding sites, allowing dsRNA editing to be localized to areas where dsRNA is produced following transcription. Genetic mutations of the Zα domain have been linked to serious neurodevelopmental disorders that are caused by the overproduction of type 1 interferons.



their environments. Herbert speculates that there could be a link between Z-DNA formation and oxidative stress in cells. During oxidative stress DNA bases themselves get oxidized, which could favor the Z-DNA conformation, with its formation acting as a sensor to activate protective pathways. "It's just a really great way of signaling that something needs to be responded to quickly, so [the cell] can then quickly assemble a complex in the right place to either repair DNA damage, or transcribe a damage response gene," he suggests. "You can actually change the genomic programming on the fly."

It has taken decades to understand that Z-DNA has significance in biology. Although there is still much to discover, it's becoming apparent that Z-DNA provides another mechanism to influence the decoding of genomic information, says Herbert. "It's pretty exciting. . . . It's a different way of thinking about the biology." ■

*Rachel Brazil is a freelance science writer from London, UK.*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## DRUGGING THE Z-CONFORMATION

Z-DNA's ability to regulate the interferon cell signaling pathway provides a route to fight a range of conditions, from viral diseases to cancers. For example, some viruses use their own Z-binding proteins to downregulate host immune responses triggered by viral RNA. This is seen in the variola virus, a member of the pox virus family that expresses the protein E3L, which mimics ADAR1 in binding to Z-DNA and in turn prevents the interferon response from ramping up against the virus (*RNA*, 20:214–27, 2014).

"In principle, blocking the viral protein, which contains the Zα domain [that binds to Z-DNA], will allow an immune response to control the virus," says Alekos Athanasiadis, who studies protein–nucleic acid interactions at the Gulbenkian Science Institute in Portugal.

Alan Herbert, founder of the DNA-based therapeutics company InsideOut-Bio, sees Z-DNA as a potential target in cancer immunotherapy. Cancer cells make a lot of double-stranded RNA, which stimulates an interferon response and tends to lead to the death of the malfunctioning cells. But some 40 percent of tumors rely on the enzyme ADAR1 to protect themselves from this response by removing those RNAs. For these tumors, inhibiting ADAR1 could prevent RNA removal and in doing so, facilitate cancer cell death, Herbert and his colleagues proposed last year (*Trends Cancer*, 5:272–82, 2019). Indeed, the deletion of ADAR1 in certain cancer cell lines causes cell death in vitro (*Nat Commun*, 9:5450, 2018).

The therapeutic potential of molecules that inhibit ADAR1 could turn out to be extremely broad, says Robert Copeland, cofounder and chief scientific officer at Massachusetts-based Accent Therapeutics. The company's most immediate focus is on treating solid tumors, says Copeland, but he foresees applications in several inflammatory diseases, including autoimmune conditions such as lupus and Crohn's disease. After developing a proprietary assay for detecting ADAR1 inhibition and screening diverse libraries of molecules, Accent researchers now have confirmed hits. "Obviously, you never know what's around the next corner, but we anticipate being able to bring an ADAR1 inhibitor into the clinic sometime in 2022—that's our ambitious objective," says Copeland.

Accent Therapeutics isn't alone in its optimism. "I know that in the Boston area, there are at least three companies really seriously looking at the role of Z-DNA in these processes and its druggability," says Herbert, who expects new drug candidates to enter preclinical testing within the next few years. He says he suspects that treatments for cancer will be the first out of the gate, but like Copeland, he emphasizes that that would be the tip of the iceberg. There is even some evidence for an alteration from the usual B-DNA to Z-DNA conformation in the hippocampus of Alzheimer's disease patients (*Neuromol Med*, 2:289–97, 2002), though he cautions that much more work needs to be carried out to fully establish what this might mean.

In the meantime, researchers continue to look for ways to target Z-DNA or induce or inhibit flipping between the right- and left-handed conformations. Last year, Kyeong Kyu Kim, a structural biologist at Sungkyunkwan University in South Korea, discovered that the antibiotic aklavin can induce Z-DNA formation (*FEBS Lett*, 593:2628–36, 2019). Kim also suggests that the bases extruded from DNA where the B and Z conformations meet could be another avenue for modulating Z-DNA formation.

Research into Z-DNA has had "a lot of fits and starts," says P. Shing Ho, a structural biologist at Colorado State University. "I think interest is going to [increase] again because of the potential link to disease states."

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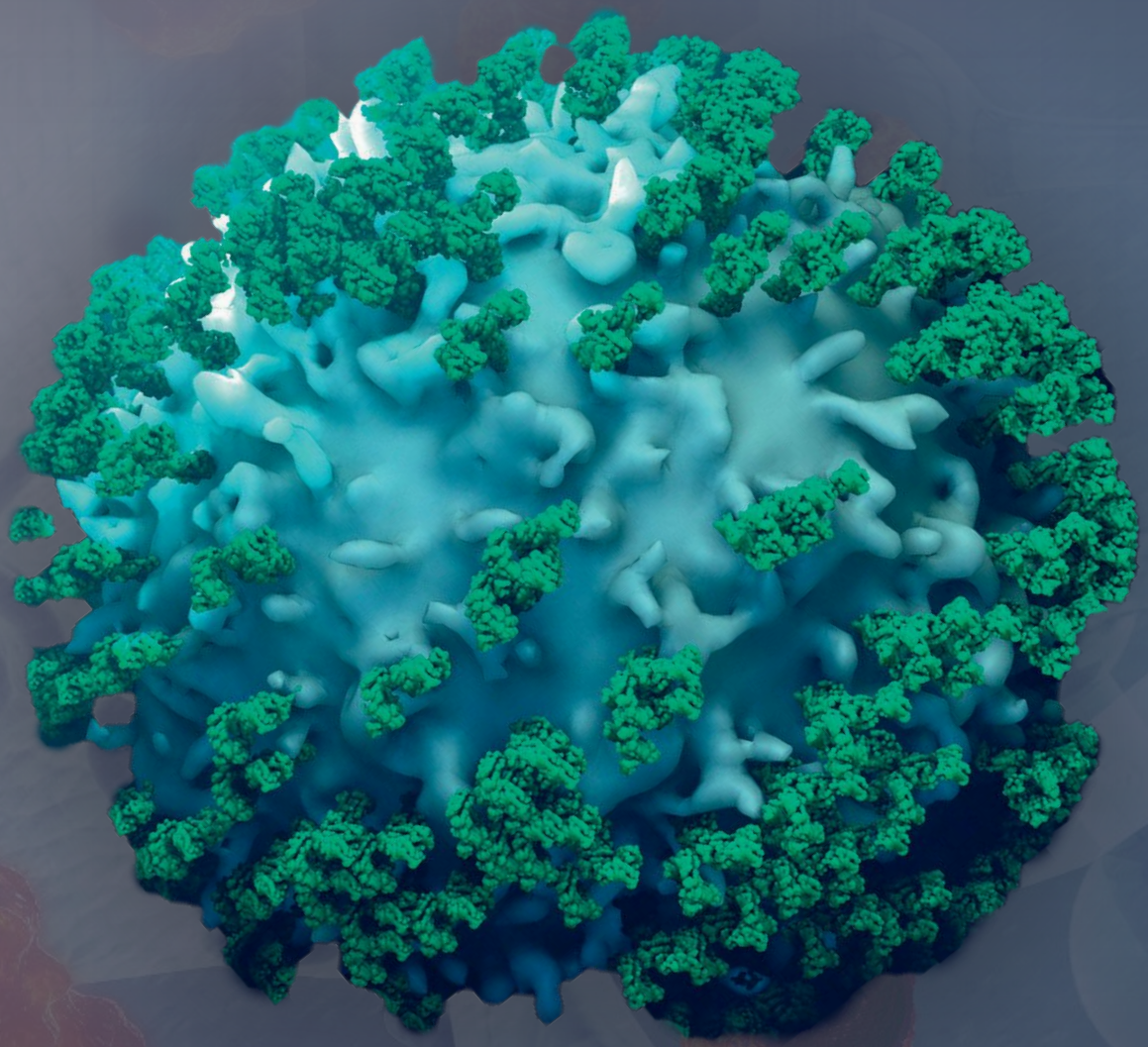
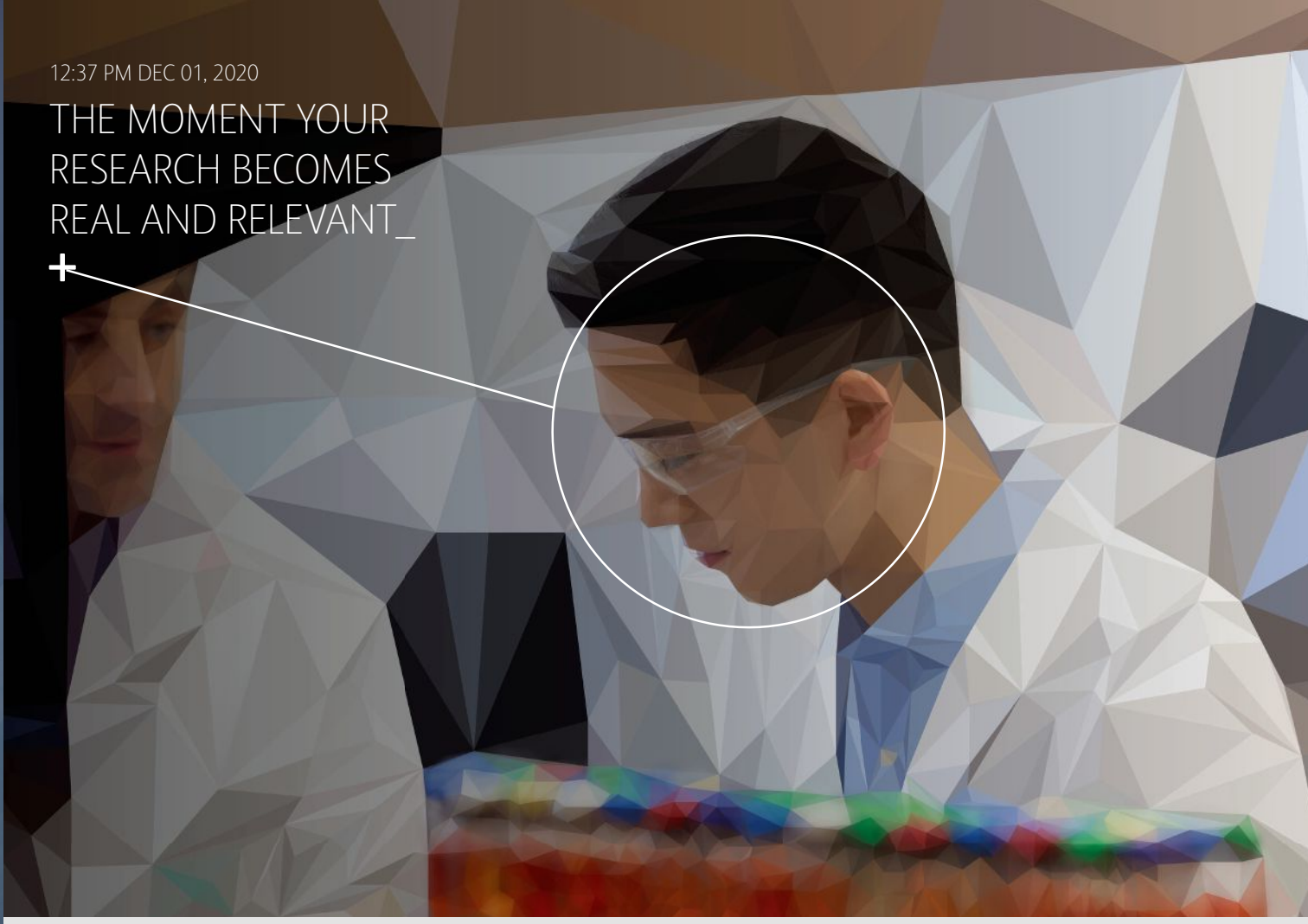


# BENCH TO BEDSIDE

## THE HISTORY OF CANCER IMMUNOTHERAPY

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# BENCH TO BED

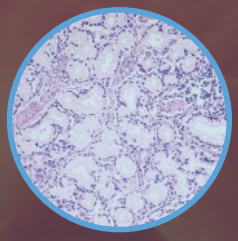
## THE HISTORY OF CANCER IMMUN

### 1890s

**1890 -** William B. Coley—now known as the father of cancer immunotherapy—administered living or dead bacteria to cancer patients. After several patients suffering from sarcoma, lymphoma and testicular carcinoma entered remission following this bacterial treatment, "Coley's toxins" became the first known "cancer vaccine[1]."

### 1900s

**1909 -** Paul Ehrlich hypothesized that tumors were immunologically different from body tissues, so host defense mechanisms should prevent neoplastic cells from developing into tumors[2].



### 1950s

**1955 -** Nicholas Mitchison showed that lymphocytes delay hypersensitivity reactions and that cellular immune reactions mediate tissue rejection, bringing lymphocytes to the forefront of immunology studies[3].

**1957 -** Richmond Prehn and Joan Main discovered tumor-specific antigens in mice[4].

**1957 -** Alick Isaacs and Jean Lindenmann discovered "viral interference" and described "type 1 interferon" (IFN)[5]. Interferons boost the immune system's response to reduce the growth of cancer.

### 1960s

**1959 -** Lloyd John Old and his colleagues showed that the tuberculosis vaccine, Bacillus Calmette-Guérin (BCG), inhibits tumors in mice[6].

**1959 -** Lewis Thomas in 1959[7] and Frank Macfarlane Burnet in 1964[8] developed the "immune surveillance" hypothesis. They suggested that lymphocytes act as sentinels to identify and eliminate somatic cells transformed by spontaneous mutations.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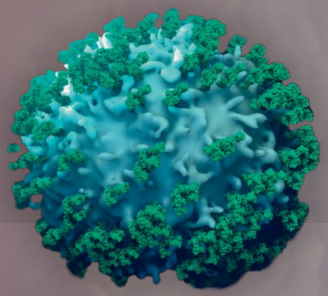
# OSIDE
# OTHERAPY



In the 19th century, a young bone surgeon
Coley, noticed that cancer patients often e
infections. This observation sparked the ide
Today, scientists have a myriad of options f
immunotherapy, and the research field is m
in immunology and cancer research have le
include adoptive cell therapy (ACT), immun
vaccines—all of which show great promise

## 1970s

**1976 -** Mitchison and colleagues identified a T-cell growth factor (now known as interleukin-2; IL-2). Produced by lymphocytes, IL-2 enabled T lymphocyte culture[9].

**1978-80 -** Several research groups purified and cloned type I IFN, leading to recombinant production of IFN-α and IFN-β[10-12].

## 1980s

**1982 -** Adoptive cell therapy (ACT) studies demonstrated that intravenously administered lymphocytes can treat bulky subcutaneous lymphomas in mice. Administering IL-2 after cell transfer enhanced the ACT therapeutic potential[13].

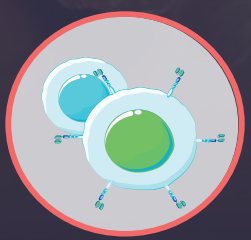
**1984 -** Researchers used interleukin-2 (IL-2) to treat the first patient with metastatic melanoma, demonstrating for the first time that immunotherapy alone stimulates T lymphocytes to ablate large invasive tumors in humans[14].

**1985 -** Researchers used IFN-α to treat patients with melanoma for the first time[15].

**1987 -** Pierre Golstein and colleagues identified cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)[16]. CTLA-4 was later identified as an immune-checkpoint molecule on T cells with potential implication in cancer immunotherapy.

**1988 -** Steven Rosenberg and colleagues used autologous tumor-infiltrating lymphocytes (TILs)—lymphocytes obtained from patients that directly oppose or surround tumor cells—to reduce metastatic melanomas in humans[17].

**1989 -** Zelig Eshhar showed that T-cell targeting receptors can be replaced, enabling researchers to direct T cells to attack any kind of cell; this was the first step towards developing gene engineered chimeric antigen receptor (CAR) T-cell therapy[18].

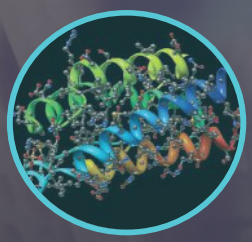


## 1990s

**1991 -** Researchers cloned and characterized the first human tumor-associated antigen from a melanoma cell line[19]. Cytotoxic T cells (CTLs) specific for a particular melanoma cell line recognize the antigen.

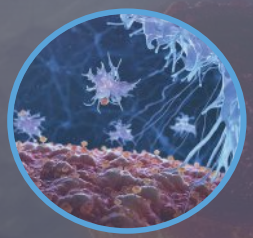
**1992 -** IL-2 received approval from the US Food and Drug Administration (FDA) for use as a metastatic renal cancer therapy based on its ability to produce durable complete responses in a small number of patients[20].

**1992 -** Yasumasa Ishida, Tasuku Honjo, and colleagues discovered and named programmed cell death protein-1 (PD-1)[21].



## 2000s

**2001 -** Researchers described cancer immunoediting—the process whereby host immune cells shape tumor fate by activating innate and adaptive immune mechanisms[22]—establishing the basis for novel individualized cancer immunotherapies.



and cancer researcher, William B.
xperienced remission following
ea of cancer immunotherapy.
for treating cancers via
ore active than ever. Great strides
d to emerging therapies which
e checkpoint inhibition, and cancer
for treating a multitude of cancers.

## 2010s

**2010 -** Sipuleucel-T immunotherapy for prostate cancer became the first US FDA-approved therapeutic cancer vaccine[23].

**2011 -** Neutralizing antibodies targeting CTLA-4 received US FDA-approval for treating melanoma[24].

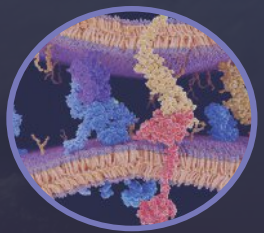
**2011 -** Antibodies targeting programmed cell death protein 1 (PD-1) received US FDA-approval for treating metastatic melanoma[25].

**2013 -** Carl June and colleagues successfully treated a refractory pediatric acute lymphoblastic leukemia (ALL) patient with CD19 CAR-T cells. This laid the foundation for subsequent US FDA approvals of CD19 CAR-T cell therapy for treatment of B-cell malignancies such as ALL and diffuse large B-cell lymphoma (DLBCL)[26].

**2015 -** Anti-PD-1 received US FDA-approval for treating lung cancer[27].

**2016 -** Anti-PD-L1 therapy for breast cancer received US FDA-approval, making it the first approved checkpoint immunotherapy for breast cancer. The drug particularly benefits patients with the aggressive triple-negative subtype of breast cancer.

**2018 -** The Nobel Prize in Physiology or Medicine was jointly awarded to James P. Allison and Tasuku Honjo for their discovery of cancer therapy by inhibition of negative immune regulation known as immune checkpoint blockade[28].



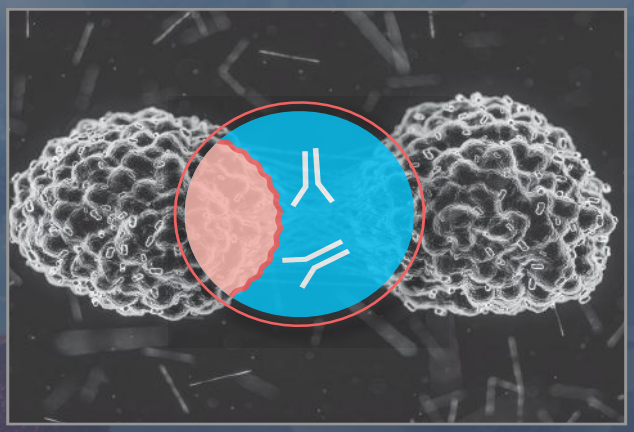
## 2020

**2020 and beyond -** Clinicians now treat more than a dozen hard-to-treat (refractory) cancers by various US FDA-approved immunotherapeutic drugs designed on varied principles such as immune-checkpoint blockade, adoptive cell therapy, CAR-T cell therapy and cancer vaccines. Future research focuses on identifying novel targets, reducing toxicity, and improving the efficacy of many cancer immunotherapies.

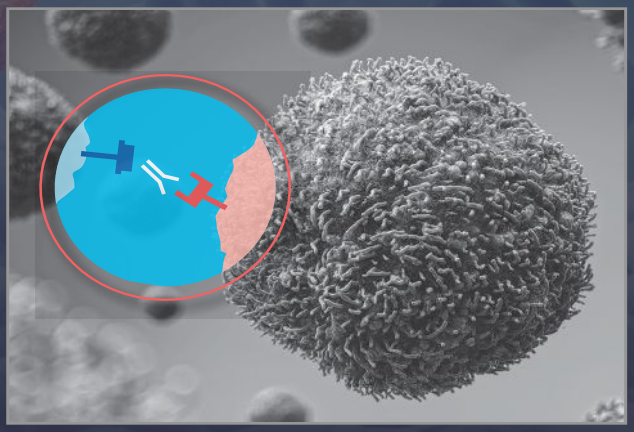
# IMMUNOTHERAPY TREATMENT TYPES

Adoptive cell transfer (ACT) uses either h
exhibiting antitumor reactivity, host cells
cally engineered with antitumor T-cell rec
(TCRs) or chimeric antigen receptors (CA
Scientists remove T cells from the patient
pand and/or genetically modify them and
them back into the patient.

### Targeted Antibodies



### Immunomodulators



BD

TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

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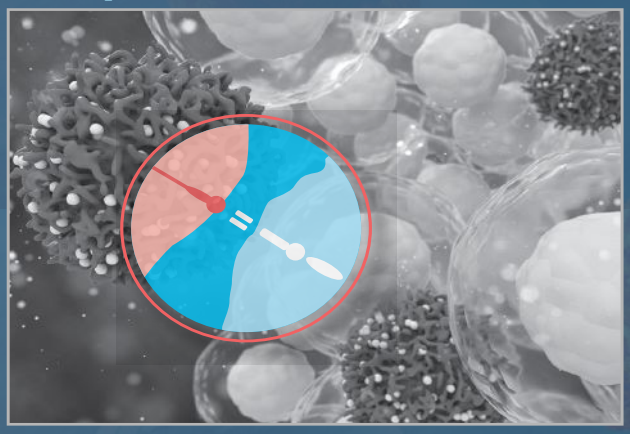
## Adoptive Cell Transfer



Adoptive cell transfer (ACT) uses either host immune cells exhibiting antitumor reactivity, host cells genetically engineered with tumor antigen specific T-cell receptors (TCRs) or chimeric antigen receptors (CARs). Scientists remove T cells from the patient, expand and/or genetically modify them, and infuse them back into the patient. Researchers are also working on novel approaches to use allogeneic third party derived immune cells with or without gene modification to use as off-the-shelf cell banks that can be applied in anti-tumor immunity.

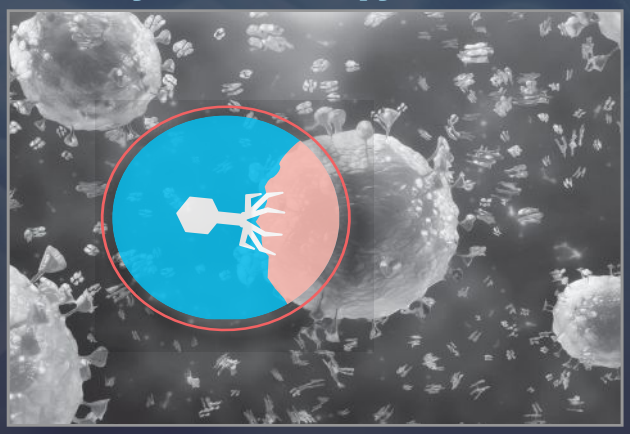
## Cancer Vaccines

Monoclonal antibodies (mAbs) target cancer antigens, causing a host immune response. Common mAbs used for cancer treatment include trastuzumab for HER2-positive breast cancer; bevacizumab for cancers with excess VEGF receptor proteins, including cervical, colorectal and ovarian cancers[29]; rituximab for types of non-Hodgkin's lymphoma with increased numbers of abnormal B lymphocytes[30]; and cetuximab for cancers with abundant EGFR protein, including colorectal cancer and some types of head and neck cancers. Scientists are currently investigating Bispecific T-Cell Engager (BiTE®) antibodies—fusion constructs linking the targeting regions of two antibodies—for cancer immunotherapy[31].

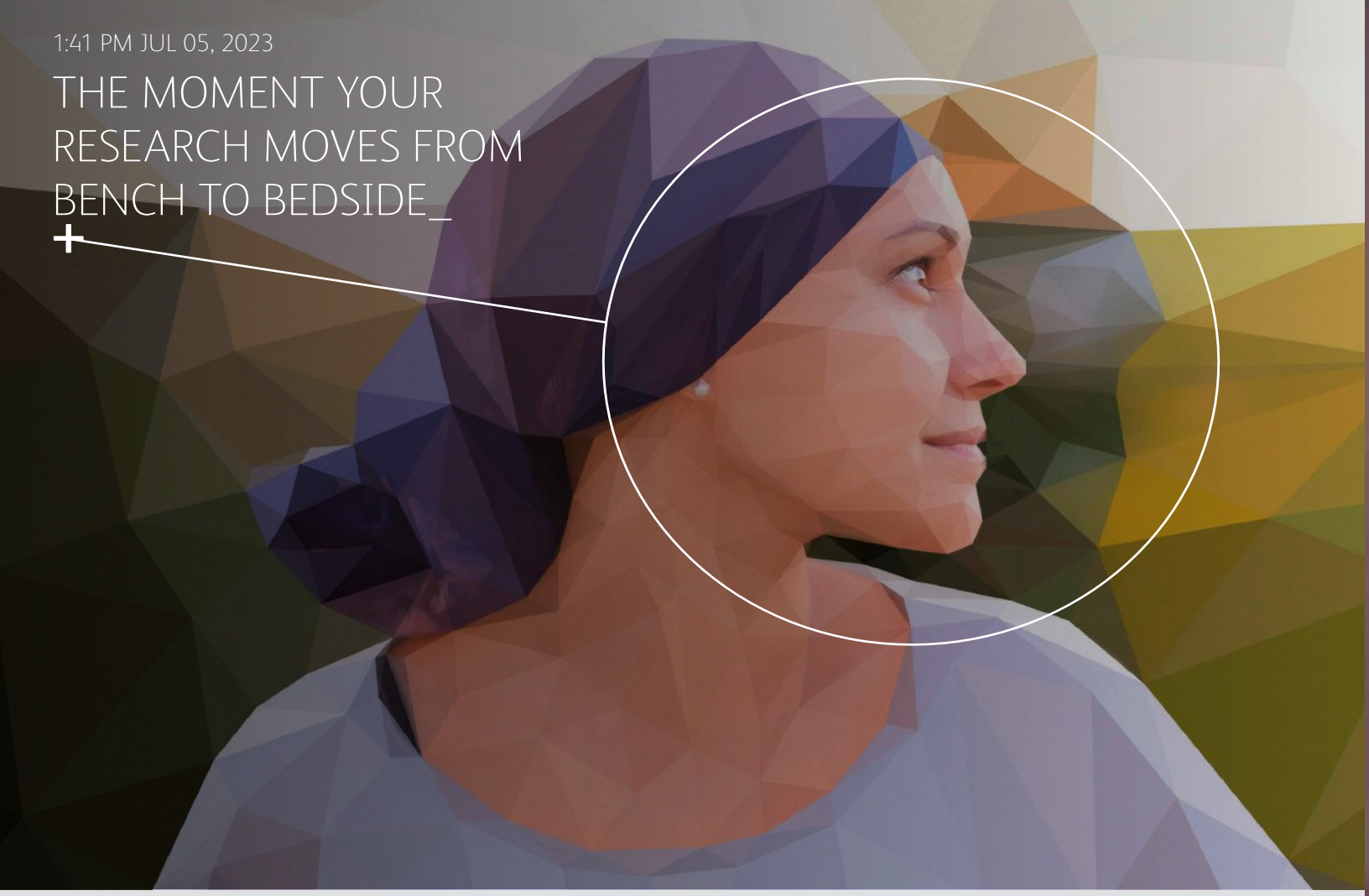
Therapeutic cancer vaccines use cancer-specific antigens to boost the body's natural defenses for fighting cancer. Scientists prepare many cancer vaccines from patient samples, with the goal of eliciting a potent, long-lasting CD4 plus CD8 T-cell expansion, which is necessary for clinical efficacy. Cancer vaccination is a developing field, with various cancer vaccines in clinical trials. Early regulatory approvals in some countries include oncophage[32], a vaccine comprising patient-extracted heat shock protein gp96 for treating kidney cancer, and sipuleucel-T[33], an immunostimulant vaccine for prostate cancer, which uses a patient's white blood cells after incubation with a prostate cancer antigen and an immune signaling factor.

## Oncolytic Virus Therapy

Immunomodulators are molecules such as checkpoint inhibitors, cytokines, agonists and adjuvants that act on pathways that regulate the immune system's activity. They block cancer cell growth signals and stimulate the body's immune system to attack cancer cells. For example, the drug thalidomide (once prescribed as an anti-nausea medication) is now used as an immunomodulator for multiple myeloma, where it boosts the immune system to fight the cancer and slows blood vessel growth around abnormal plasma cells.



Oncolytic viruses can infect and replicate in tumor cells without harming normal tissues. Globally, there are three approved oncolytic viruses: an adenovirus for treating advanced head and neck cancer, an oncolytic reovirus approved for treating advanced melanoma, and an oncolytic herpes simplex virus approved for treating advanced melanoma[34].

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# Baby's Microbial Garden

The gut microbiome is particularly malleable in the first two years after birth, allowing probiotics to make their mark. Can we exploit this to improve infants' health?

BY JENNIFER T. SMILOWITZ AND DIANA HAZARD-TAFT

In the fall of 2018, a team of researchers from the Weizmann Institute of Science in Israel published findings that a cocktail of 11 strains of *Lactobacillus* and *Bifidobacterium* had minimal immediate impact and no lasting effect on the makeup of the gut microbiome of mice or people. In fact, the probiotic bacteria were not found in any of the fourteen adult participants after supplementation ended.[1]

These recent findings received quite a lot of press and added to growing sentiment among the public that probiotics—live microorganisms that are purported to confer benefits on the human host—don't work. Decades of research have shown that most probiotics aren't able to colonize or exert lasting benefits in the human gut. Some critics even suggested that probiotics may not be a promising avenue for treating disease or otherwise improving health and wellness. But we thought: "Don't throw the baby out with the bathwater—our work shows that the right probiotic can work in the infant gut." Findings we published in 2017 showed that feeding breastfed babies a probiotic that included a specific strain of *Bifidobacterium longum* subspecies *infantis* (*B. infantis* EVC001) resulted in a 10,000,000-fold average increase in levels of fecal *B. infantis*. This level persisted for one month after the supplement was consumed,[2] and levels remained elevated for up to one year after treatment.

Colonization of the infant gut by *B. infantis* had protective effects, such as lower levels of potential gut pathogens and fecal endotoxin, an outer membrane component of Gram-negative organisms known to trigger inflammation.[2] We also found that infants given the *B. infantis* probiotic had reduced intestinal inflammation compared with breastfed infants who did not receive the probiotic.[3] The gut microbiomes of *B. infantis* supplemented babies harbored fewer antibiotic resistance genes—a sign of fewer pathogens[4]—and showed less degradation of mucin, a glycoprotein secreted by the intestinal epithelium that protects epithelial cells from direct contact with gut microbes.[5] These data support earlier findings from Mark Underwood and colleagues at the University of California, Davis. In 2013, Underwood's team showed that feeding preterm infants a different strain, *B. infantis* ATCC15697, resulted in greater increases in fecal *Bifidobacterium* and reduced levels of potential pathogens compared with infants given a probiotic containing *B. lactis*.[6]

To understand why the infant gut microbiome changed so drastically over the past century, we sought to understand how the infant gut microbiome forms.

While the scientific community and the public grappled with repeated findings that probiotic supplements taken by adults are not consistent in effectively colonizing the gut or conferring benefit, we now had convincing evidence that babies' gut microbiomes responded incredibly well to specific strains of *B. infantis*. The question was why.

## Microbiome origins

Hints about the infant microbiome can be found in century-old articles on commensal bacteria in infant feces. W. R. Logan, a clinical pathologist at the Research Laboratory of the Royal College of Physicians in Edinburgh, was the first to report, 100 years ago, that bacteria in fecal smears from breastfed infants were a near monoculture of *Bacillus bifidus*,[7] which is today known as the genus *Bifidobacterium*. Fecal smears from formula-fed infants of that time, by contrast, had a diversity of bacteria, with relatively few *Bifidobacterium*—more similar to the microbial diversity found in today's breastfed infants.

These striking changes in the gut microbiome composition seen over the past century were consistent with our recent finding that the fecal pH in breastfed infants dramatically increased from pH 5.0 to 6.5 within the past 100 years, a change associated with an apparent generational loss of *Bifidobacterium* and concomitant increase in potential pathogens.[8] The reduction in *Bifidobacterium* in the gut microbiome of breastfed infants is likely an unintended consequence of medical practices that can save lives but do not support the growth of *Bifidobacterium*. Such medical practices include treatment with antibiotics to which *Bifidobacterium* are sensitive; infant formula that doesn't provide the specific food the bacterium requires; and greater numbers of cesarean section deliveries, which bypass the route by which the bacterium is transferred from mother to baby. These medical practices have been implicated in the increased risk for allergic and autoimmune diseases prevalent in resource-rich nations. The reduction in *Bifidobacterium* and increase in proinflammatory microbes in early infancy is proposed to occur during the critical window of immune system development, and thereby may increase the risk for immune disease later in life.[9]

To understand why the infant gut microbiome changed so drastically over the past century, we sought to understand how this community forms. Infant gut microbiome colonization begins at delivery with exposure to maternal microbes—mostly vaginal and fecal microbes for vaginally delivered babies or predominately microbes from the skin, mouth, and surrounding environment in infants born by cesarean delivery. After birth, infants are bombarded by a vast array of microbes found in the environment, including in breast milk, but the species that go on to become durable members of the microbial community are often those transmitted by the infants' mothers through physical contact.

Children continue to acquire gut microbiome species from their mothers and others in the community during early life. This stands in contrast to an adult's gut microbiome, which is stable and resists change largely because the available space and food is already used by established microbes—the ecological niches are simply occupied in adult guts. Thus, it makes sense that a probiotic has a better chance of persisting in the infant gut, where it faces less competition, and therefore is more likely to have food it can consume and a location where it can grow. A probiotic serves as just one more source of exposure to new bacteria for the infant.

Recognizing this, we began to wonder: In our studies, what ecological niche did *B. infantis* fill that supported its persistence in infants long after probiotic administration stopped?

## Setting the stage

A major factor in determining which bacteria thrive in the gut is the availability of their carbohydrate food sources. Thus, for a probiotic to work in an infant, microorganisms should be selected so that the food source they use most efficiently matches what's available—a food that is present and not already being consumed by other bacteria. We set out to determine what carbohydrates *B. infantis* consumes in the infant gut.

Naturally, we turned to breast milk, which for millions of years has been the single food that can exclusively nourish and protect babies for the first six months of life. Human milk

delivers nutrients as well as non-nutritive, bioactive molecules, including carbohydrates known as human milk oligosaccharides (HMOs). Back in the mid-1900s, Paul György, a world-renowned biochemist, nutritionist, and pediatrician from the Hospital of the University of Pennsylvania, and colleagues unknowingly referred to HMOs when they proposed the existence of a "bifidus factor," something unique in breast milk that fed *Bifidobacterium*.[10] While humans cannot digest HMOs, it turns out that *Bifidobacterium*, especially *B. infantis*, can. In 2007, our group at UC Davis used mass spectrometry–based tools coupled with microbiology to show that *B. infantis* gobbles up HMOs as its sole energy source, while other species of *Bifidobacterium* consume only some HMOs[11] in addition to plant-, animal-, and host-derived carbohydrates.[12]

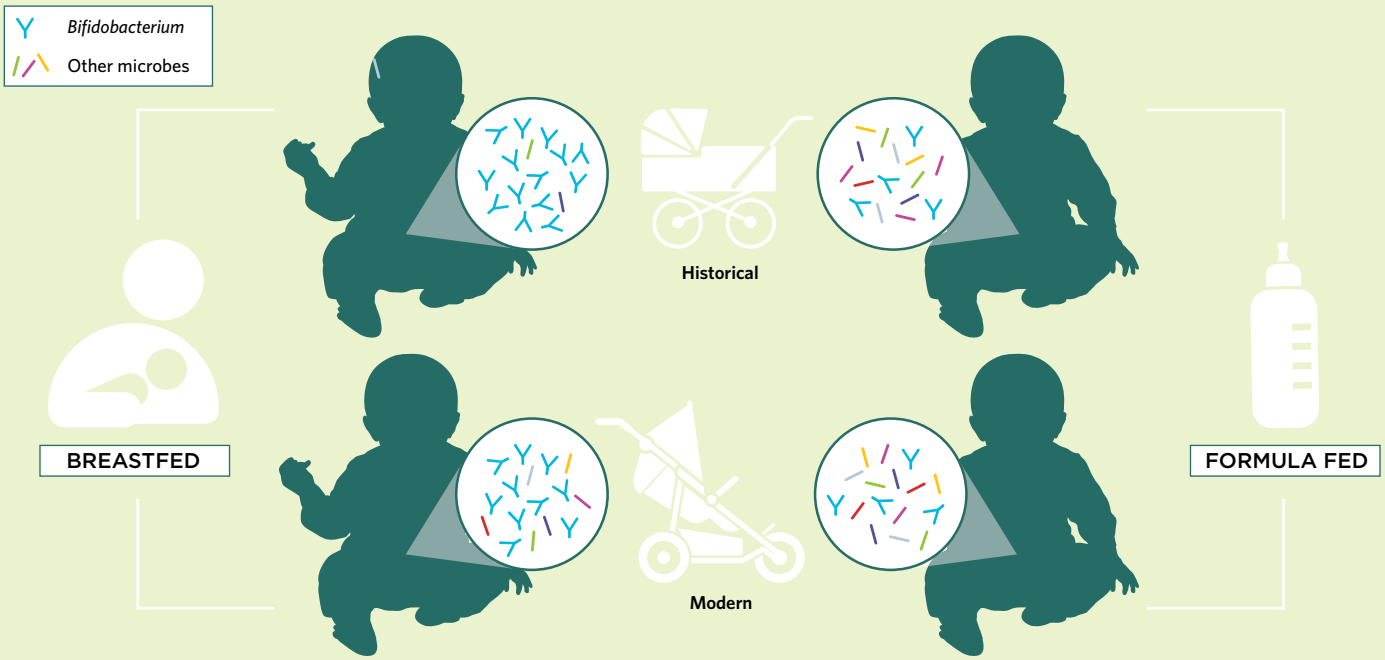
HMOs are a diverse class of complex carbohydrate molecules synthesized by the mammary gland. With approximately 200 different molecular species, they represent the third most abundant solid component in human milk following lactose and fat. Because HMOs are complex and vary in structure, they are expensive to manufacture. Current infant formulas may contain one or two simple HMO structures, but at a fraction of the concentration found in breast milk. Infant formulas lack the abundance and complexity of HMOs to selectively feed beneficial gut microbes and to bind and neutralize pathogens from the gut.

The bacterial species in the infant gut capable of consuming HMOs can be considered the milk-oriented microbiome (MOM). Although *B. infantis* appears to be the most efficient consumer of HMOs, other species of *Bifidobacterium*, in particular, *B. breve* and *B. bifidum*, can and do consume some HMOs but also consume plant-, animal-, and host-derived carbohydrates. The *Bifidobacterium* species that colonize the gut change throughout life in response to available carbohydrates in the host diet. For instance, *B. infantis*, *B. breve*, and *B. bifidum* are MOM bifidobacteria typically found in the stool of exclusively breastfed infants, while *B. longum* and *B. adolescentis*, which preferentially consume plant- and animal-derived carbohydrates, are typically found in the stool of adults. Yet there is variation and overlap in the species present at different life stages.[12]

Of the MOM bifidobacteria found in the infant gut microbiome, different species may have different implications for the microbiome. For example, when we gave exclusively breastfed infants a supplement with the probiotic *B. infantis* EVC001, their gut became dominated by the genus *Bifidobacterium*—upwards of 80 percent relative abundance of the gut microbiome—and potential pathogens made up less than 10 percent

## THE CHANGING INFANT MICROBIOME

Historically, the breastfed infant gut microbiome was a near monoculture of *Bifidobacterium* (*J Pathol Bacteriol*, 18:527–51, 1913). The formula-fed infant gut microbiome was much more diverse. The breastfed infant gut microbiome and the formula-fed infant gut microbiome are now more similar to the historical formula-fed infant gut microbiome, although modern breastfed infants do have more *Bifidobacterium* than modern formula-fed infants.



of the community. On the other hand, the gut microbiomes of exclusively breastfed infants who were not supplemented with *B. infantis* EVC001 had much lower levels of *Bifidobacterium*, with only about 30 percent relative abundance, and potential pathogens constituted about 40 percent of the microbes in their gut,[2] findings that are consistent with previous work from our group and others.[13,14] This near-monoculture of *Bifidobacterium* appeared to be driven by *B. infantis*, which represented about 90 percent of the total *Bifidobacterium* in infants fed the probiotic. In contrast, *B. longum* was the predominant gut *Bifidobacterium* in the control group, followed by *B. breve* and *B. bifidum*.[4] These data highlight the vital importance of strain specificity in probiotics, and the combination of the presence of *B. infantis* and breastfeeding to support a protective gut environment in infants.

To understand how supplementary *B. infantis* can so successfully outcompete other microbes in the infant gut, we took a deep dive into its feeding strategy. Turns out it is a picky eater, exclusively dining on HMOs, and when HMOs are abundant, *B. infantis* gobbles them up ravenously. Unlike other MOM bifidobacteria, *B. infantis* possesses all the genes necessary for the complete, internal degradation of HMOs and preferentially uses HMOs over any other carbohydrate source. Other MOM bifidobacteria such as *B. bifidum* and *B. breve* strains display growth capabilities with only a subset of HMOs.[15,16] *B. infantis* thus has a competitive advantage when breast milk makes up the entire diet.

A major factor in determining which bacteria thrive in the gut is the availability of its carbohydrate food source.

A 2008 study from colleagues at UC Davis and their collaborators showed how *B. infantis* makes quick use of HMOs: with binding proteins to grab HMOs from the gut lumen and transporters to usher them into the cytoplasm, breaking them down into monosaccharides that are then fermented into lactate and the short-chain fatty acid acetate that are secreted from the cell.[17] These end products maintain a lower pH in the intestinal milieu, supporting the transport of these compounds into the intestinal epithelium for use by the host and creating an undesirable environment for potential pathogens. The production of acetate also blocks the infiltration of toxic molecules produced by pathogenic bacteria by enhancing intestinal barrier function and inhibiting pro-inflammatory and apoptotic responses.[18] Recent findings from one in vitro study have shown that the amount of acetate and lactate produced by different bifidobacterial species is dependent on how well they consume the carbohydrates available to them. Hence, feed a carbohydrate-consuming microbe its preferred carbohydrate, and it has greater potential to produce more of its protective end-products.

Another reason why *B. infantis* outcompetes other bifidobacterial strains in the gut of breastfed infants is that all of its HMO digestion happens inside the bacterial cell. *B. bifidum*, on the other hand, digests HMOs externally. This extracellular digestion liberates simple carbohydrates and may cross-feed other species of *Bifidobacterium*,[12] but also cross-feeds and thus opens an ecological niche for other, perhaps less beneficial microbes. Cross-feeding among microbes diversifies the gut microbiome, which is considered to be generally beneficial in adults.

But is there an advantage to having a near monoculture of *Bifidobacterium* in infants? By asking this question, our focus turned to immune development.

## Benefits of a *Bifidobacterium*

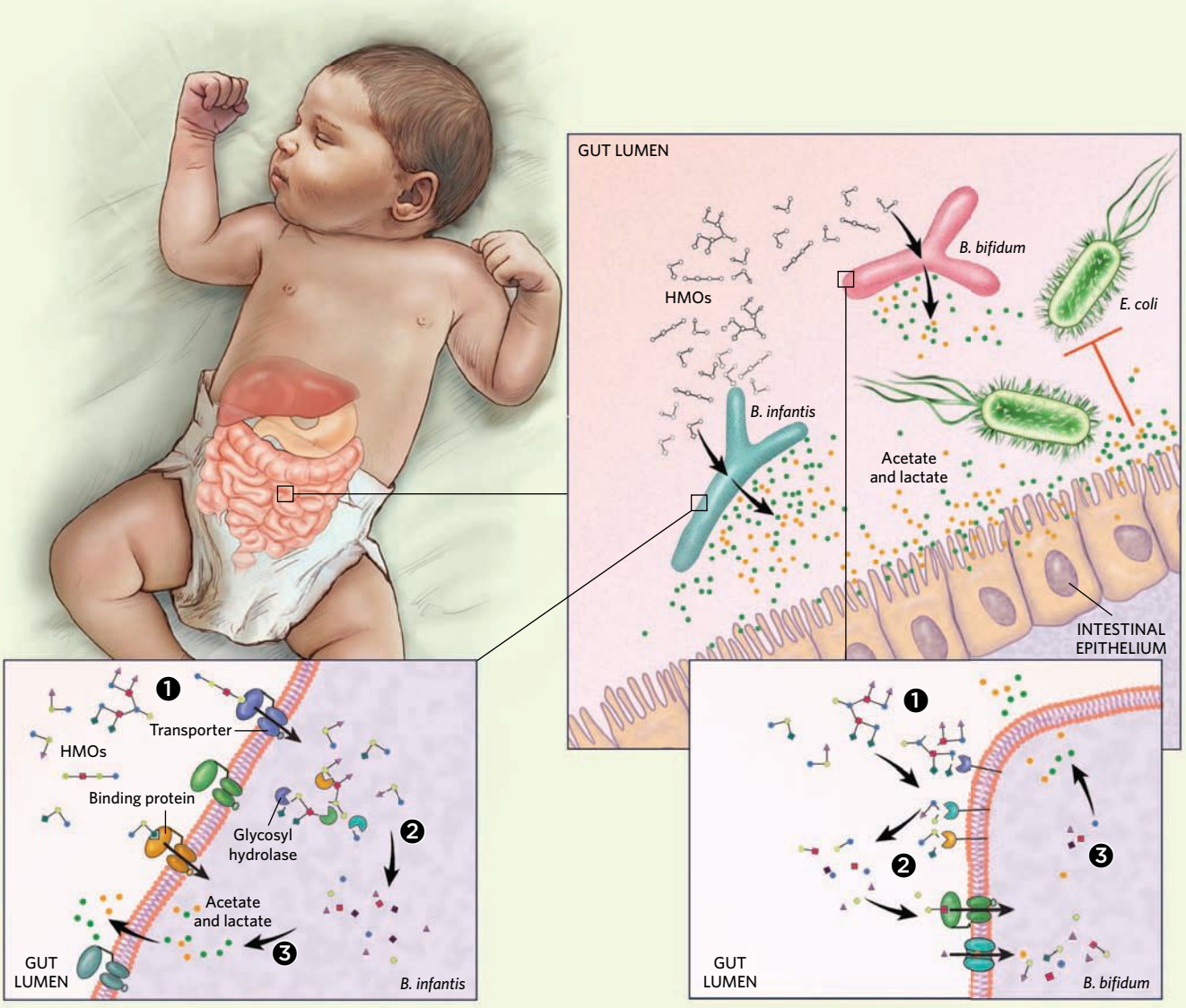
The decline of *Bifidobacterium* in infant gut microbiomes and the associated dysregulation of the microbial community, with more numerous potential pathogens, has been suggested as one possible contributor to the increased incidence of autoimmune diseases that plague residents of resource-rich nations. Conversely, observational studies have shown beneficial immune effects of having a fecal microbiome dominated by *Bifidobacterium*. In two studies in Bangladeshi infants and young children, fecal *B. infantis* and *Bifidobacterium* abundances at two months of age were strongly correlated with improved vaccine responses at six months and two years old compared with infants not colonized by *B. infantis* or with low relative abundances of *Bifidobacterium*.[19,20]

Additionally, bifidobacteria are less likely than other microbes, especially potential pathogens, to carry and share antimicrobial resistance genes, which can lead to a higher risk of antibiotic-resistant infections. In an observational study of Bangladeshi and Swedish infants, a dominance of intestinal *Bifidobacterium* was associated with a significant reduction in both the number and the abundance of antibiotic resistance genes.[21] Moreover, compared with matched-control breastfed infants, supplementation with *B. infantis* EVC001 led to a reduction of antibiotic resistance genes by 90 percent, a drop largely driven by a reduction in levels of *Escherichia*, *Clostridium*, and *Staphylococcus*—potentially pathogenic bacteria that play a major role in the evolution and dissemination of antibiotic resistance genes.[4]

In an effort to restore the *Bifidobacterium*-dominated infant gut microbiome that was typical of breastfed babies 100 years ago, we decided to conduct a randomized, controlled trial using the *B. infantis* EVC001 probiotic. Given that not all *B. infantis* strains consume all HMOs efficiently,[22] we selected *B. infantis* EVC001 because we knew this strain had the full cas-

# THE MILK-ORIENTED MICROBIOME

Human milk oligosaccharides (HMOs) are complex carbohydrates that microbial species of the milk-oriented microbiome (MOM) can use as a food source. *Bifidobacterium infantis* encodes many proteins that specifically bind and transport all types of HMOs into its cell and digest them internally. Other *Bifidobacterium* species digest only some HMOs and some do so externally. Digestion of HMOs by MOM *Bifidobacterium* results in the production of lactate and the short chain fatty acid acetate, that are secreted into the gut lumen. These molecules lower the pH in the intestinal milieu, which improves their transport into the epithelium for use by the host and creates an undesirable environment for potential pathogens such as *E. coli*.




***B. INFANTIS*** **PREFERENTIALLY CONSUMES ALL HMO SPECIES OVER ANY OTHER CARBOHYDRATE SOURCE.**

❶ Binding proteins glom on to HMOs and usher the carbohydrates to transporters that move them into the bacterial cell.

❷ Intracellular glycosyl hydrolases cleave each glycosidic linkage of all HMO structures, yielding monosaccharides.

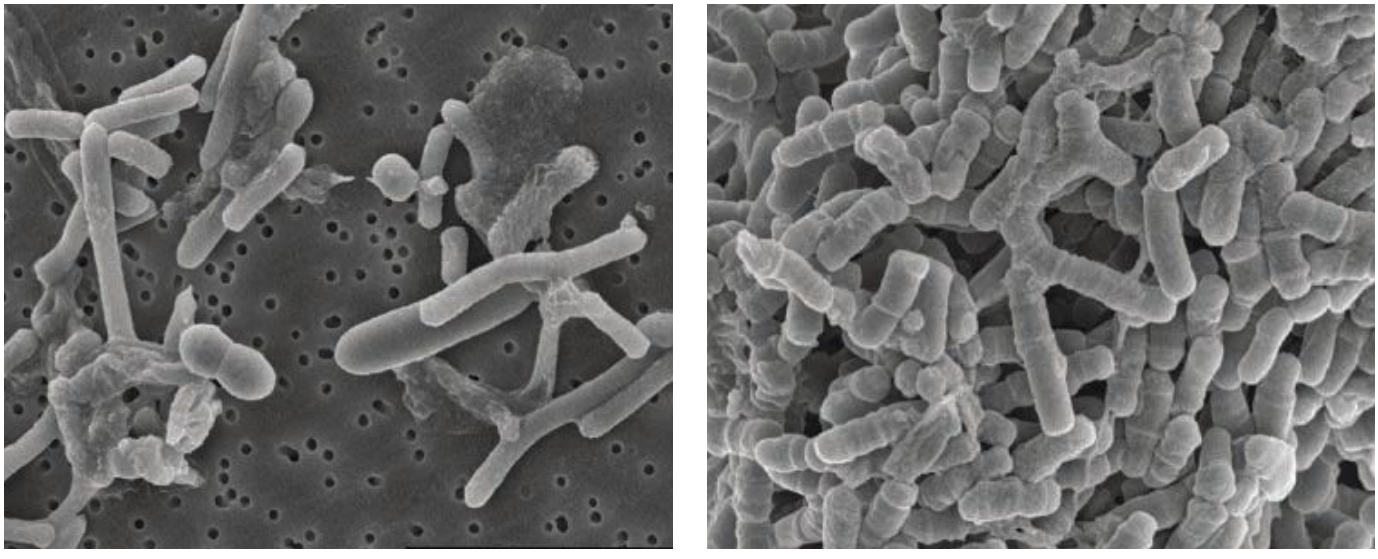
❸ These monosaccharides are metabolized into acetate and lactate that are secreted from the cell.

***B. BIFIDUM*** **EATS ONLY A SUBSET OF HMOs.**

❶ Glycosyl hydrolases attached to the outer cell membrane break down HMOs into mono- and disaccharides in the extracellular space.

❷ These molecules are imported via transporters, and some are gobbled up by other intestinal microbes, a process called cross-feeding.

❸ The mono- and disaccharides are further metabolized into acetate and lactate, though because *B. bifidum* is a less efficient consumer of HMOs, it likely produces less of these products than *B. infantis*.



A PROBIOTIC THAT STICKS: Scanning electron micrographs of infant fecal samples show a large increase in the number of *Bifidobacterium* microbes in those treated with a probiotic called EVC001 (right) compared with controls (left).

sette of genes needed to fully digest all HMOs. Healthy, full-term, breastfed infants were randomized to consume *B. infantis* EVC001 for 21 consecutive days starting on day 7 postnatal or to not receive the probiotic.

Compared with breastfed control infants who did not receive the probiotic, supplementation resulted in a 10,000,000-fold average increase in levels of fecal *B. infantis* and increased fecal *Bifidobacterium* by 79 percent during the supplementation period, and this was still true at one month post supplementation. This means *Bifidobacterium* colonization persisted without the continuation of probiotic supplementation. Additionally, colonization of *B. infantis* persisted until one year of age if infants were continuing to consume any breast milk and were not exposed to antibiotics. Importantly, the supplemented infants exhibited an 80 percent reduction in potential gut pathogens belonging to the families *Enterobacteriaceae* and *Clostridiaceae* and reduced fecal endotoxin. Additionally, we saw a 2-fold increase in fecal lactate and acetate and a 10-fold decrease in fecal pH. The supplemented infants' gut microbiomes and biochemistry resembled norms observed a century ago.

We also identified some clues about the consequences of the gut microbiome's "modernization." Breastfed infants with low fecal *Bifidobacterium* had excreted 10-fold more HMOs in their stool throughout the two-month study period than infants supplemented with *B. infantis* EVC001, indicating that HMOs—the third most abundant component in breast milk—were going to waste. We also found that infants with low fecal *Bifidobacterium* had several-fold higher levels of fecal proinflammatory cytokines compared with infants whose gut microbiomes were dominated by *Bifidobacterium* post supplementation with *B. infantis* EVC001.[3]

Taken together, these data demonstrate that this particular strain of *B. infantis*, provided as a probiotic to breastfed infants, dramatically colonized the infant gut microbiome during and after supplementation, and beneficially remodeled the microbial, biochemical, and immunological environment in the infant gut. Many infants around the world never acquire *B. infantis*, but the combination of breastfeeding and probiotic supplementation with this bacterium seems to lead to a nourishing and protective gut environment.

Our findings also support the hypothesis that the ineffectiveness of some probiotics in adults is due in part to the fact that they are introducing a new species to an established community with few ecological niches still open. Probiotics may not work in infants when there is a mismatch between the carbohydrate needs of the probiotic and the availability of highly specific carbohydrates such as HMOs in breast milk. Because *B. infantis* efficiently consumes almost all HMOs found in breast milk, it is likely to find an open ecological niche and then outcompete other microbes, especially proinflammatory pathogens.

Many scientists are working to understand what the infant gut microbiome really means for health across the lifespan. Meanwhile, we are turning our attention to other questions: How do colonization patterns of *Bifidobacterium* differ in infant populations around the world from infancy to weaning? And what solid foods support a healthy gut and immune system? Working with funding from the National Institutes of Health, we are now conducting a study designed to understand how the carbohydrate structures of complementary foods influence microbial function that will support a healthy gut microbiome and immune system development in late infancy and early toddlerhood. The ultimate goal is to identify specific

*PEDIATR RES*, 86:749–57, 2019

Many infants around the world never acquire *B. infantis*, but the combination of breastfeeding and probiotic supplementation with this bacterium seems to lead to a nourishing and protective gut environment.

carbohydrate structures in the diet that selectively feed beneficial gut microbes in children during the critical window of immune development for lifelong health. ■

*Jennifer Smilowitz is the associate director of the Human Studies Research Program at the Foods for Health Institute and a research scientist in the Department of Food Science and Technology at the University of California, Davis. Diana Taft is a postdoctoral research fellow in David Mills's lab in the Department of Food Science and Technology and a member of the Foods for Health Institute at UC Davis.*

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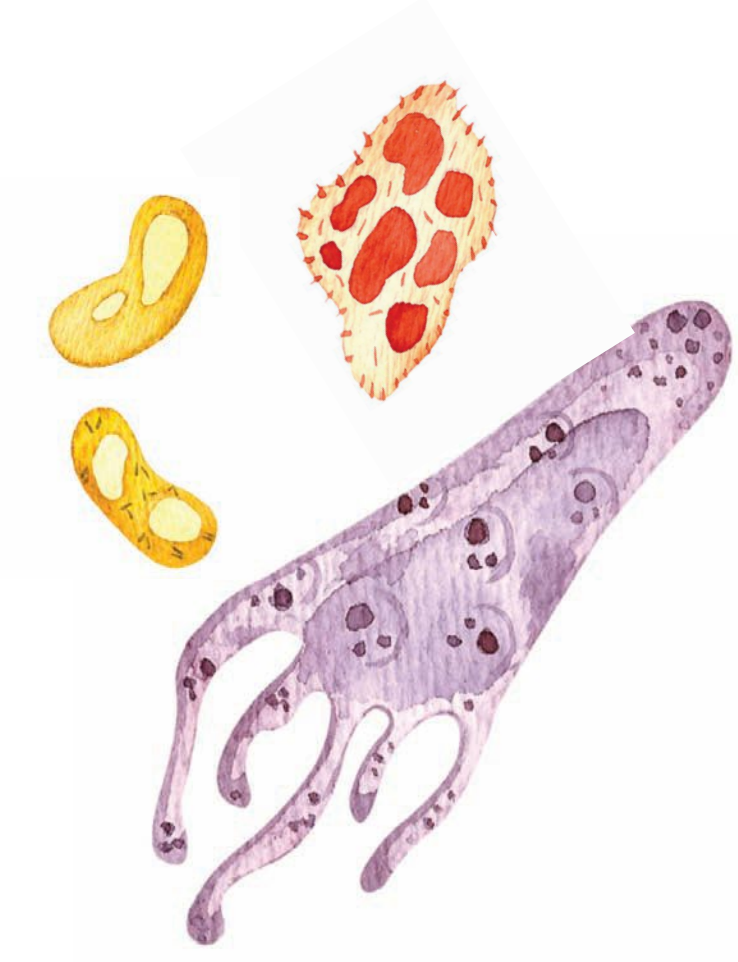


# The Literature

IMMUNOLOGY

## Toxin Sponges

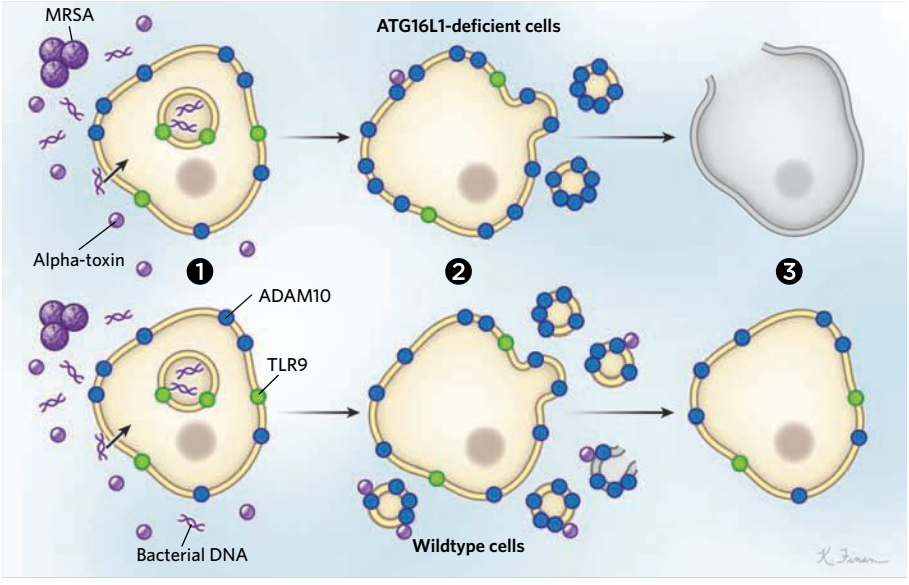
THE PAPER

M.D. Keller et al., "Decoy exosomes provide protection against bacterial toxins," *Nature*, 579:260–64, 2020.

To kill a cell, methicillin-resistant *Staphylococcus aureus* discharges an arsenal of toxins. Alpha-toxin is one of its favorites, forming a cylinder of sharp peptides that punches a hole in the host cell, popping it. MRSA is extremely virulent and resistant to many antibiotics, leading scientists to search for ways to defeat it.

Bacteriologist Victor Torres and cell biologist Ken Cadwell, both of New York University Grossman School of Medicine, had found in previous work that a key protein involved in autophagy also makes mice less susceptible to MRSA and alpha-toxin. But what was the connection between autophagy and MRSA infection? To find out, Torres, Cadwell, and graduate student Matthew Keller depleted the autophagy protein ATG16L1 in a human cell line and found that the cells then displayed unusually high numbers of a transmembrane protein called ADAM10 on their surfaces. Alpha-toxin binds to ADAM10 in order to puncture the cell, and when these cells were treated with alpha-toxin, they were more likely to die than control cells that had normal levels of ATG16L1.

Further experiments revealed that ATG16L1 and other autophagy proteins regulated the cultured cells' release of exosomes, small bubbles of cytosol wrapped in plasma membrane and studded with the cell's own proteins. The presence of bacteria, particularly their DNA, was necessary for the release of exosomes from the cell, the team found. When the cultured cells had normal levels of autophagy proteins and were exposed to bacterial DNA, they released far more exosomes laden with ADAM10 than did the ATG16L1-deficient cells.




**PUNCTURE THIS:** Based on experiments with mice and cultured human epithelial cells, researchers pieced together how hosts can use exosomes to defend themselves against bacterial toxins. Host cells detect the presence of bacteria using a receptor called TLR9, which senses bacterial DNA ❶. In wild-type mice, host cells respond by releasing exosomes studded with the protein ADAM10, which act as decoys, but mice deficient in an autophagy protein called ATG16L1 release far fewer of the decoy exosomes ❷, indicating the autophagy protein is necessary to mount an effective exosome response. Indeed, while MRSA's alpha-toxin binds the host receptor ADAM10 on exosomes in wildtype mice, destroying the vesicles, in mice lacking ATG16L1, alpha-toxin binds ADAM10 on host cells, forming a pore and killing cells ❸.

Western blots for alpha-toxin showed that the compound bound preferentially to exosomes from ATG16L1-rich animals that had ADAM10, and further experiments demonstrated that decoy exosomes worked in mice as well: exosomes from wild-type mice, but not those from ATG16L1-deficient mice, prolonged survival when transferred into MRSA-infected mice. Exosomes also protected other cells from two additional bacterial toxins, leukotoxin ED and diphtheria toxin.

The team found that the innate immune receptor TLR9 senses bacterial DNA in human and mouse cells and triggers exosome release, although the team hasn't yet determined all the steps in the pathway. "The fact that triggering that innate immune pathway leads to production of exosomes makes us fairly confident that this isn't some accident—this is the host defense response," says Cadwell.

The work is "one of the more extraordinary papers that I've seen," says emeritus cell biologist Philip Stahl of Washington University School of Medicine in St. Louis who reviewed the study for *Nature*. "It's really uncovering new territory in exosome biology."

Whether exosomes could be used in the future as a treatment to minimize damage during an infection remains to be seen. Also worth exploring, Torres and Cadwell say, are the possible effects on infection resistance of a human ATG16L1 variant that's common in the population.

—Rachael Moeller Gorman

**WHAT'S IN A WING?:** Male pea aphids (*Acyrthosiphon pisum*) come in winged and wingless morphs, a phenotype determined by an autosomal insertion on the X chromosome.

**A SOCIABLE GUT:** A study uncovers connections between gut microbes such as *Bifidobacterium* (illustrated above) and people's social lives.

DEVELOPMENTAL BIOLOGY

# To Wing or Not to Wing

**THE PAPER**

B. Li et al., "A large genomic insertion containing a duplicated follistatin gene is linked to the pea aphid male wing dimorphism," *eLife*, 9:e50608, 2020.

The sap-sucking pea aphid (*Acyrthosiphon pisum*) has both winged and wingless morphs. When pea aphid mothers are raised with abundant food, their asexually produced daughters develop no wings, but in crowded, food-scarce conditions, daughters are born with wings, which helps them find better living conditions. In males, which are the product of sexual reproduction, whether or not they grow wings appears to be genetically controlled. "So you've got one species, two dimorphisms," explains Jennifer Brisson, an evolutionary geneticist at the University of Rochester in New York. "One is totally plastic, and the other is totally genetic."

Brisson's team discovered that the male wingless morph is controlled by an insertion in one version of the X chromosome, the only sex chromosome in pea aphids: males whose single X chromosome carries the insertion fail to grow wings, while those that lack it develop the appendages. Brisson suspects that a duplicated *follistatin* gene on the insertion may play a role. The original *follistatin* gene, which resides on an autosome, encodes a glycoprotein that regulates the expression of the ecdysone receptor, whose signaling is involved in wing development in females.

The winged and wingless forms seem to be actively maintained in at least two of the 15 or so closely related variants within the pea aphid species complex, suggesting that each morph has a fitness advantage, depending on context. "The thing I find most interesting is the evidence that the polymorphism has been segregating in aphids for such a long time, becoming fixed or lost in some lineages, but remaining at play in at least the pea aphid lineage for perhaps 10 million years," says David Angelini, a biologist who studies insect polymorphisms at Colby College in Maine but was not involved in the study.

—Viviane Callier

MICROBIOLOGY

# Microbes and Your Behavior

**THE PAPER**

K.V.-A. Johnson, "Gut microbiome composition and diversity are related to human personality traits," *J Hu Mic*, 15:100069, 2020.

Researchers have shown that fecal transplants in mice can change the animals' temperaments. Several studies have also linked the human microbiome to psychiatric illnesses, including autism and depression. But to date, few experiments have considered the microbiome of the general population and whether variations in gut bacteria are associated with personality traits, says microbiome-gut-brain axis researcher Katerina Johnson of Oxford University.

In a recent study, Johnson analyzed gut microbiome data obtained from stool samples of 655 individuals, along with survey-based information about their personality and behavior, health and lifestyle, dietary habits, and sociodemographics. She found that people who have larger social networks are more likely to have greater gut microbiome diversity, which research indicates is associated with both gut health and general health. The analysis also showed that "sociable people tend to have a higher abundance of certain types of gut bacteria" that have been found to be less abundant in people with autism, Johnson says. She adds that her analysis also identified bacteria found in lower abundances in sociable people that had previously been found to be highly abundant in autistic people.

She notes that further research is needed to directly investigate any effect that gut bacteria may have on human behavior, but ultimately, she says, these findings and follow-up research "might help with the development of new therapies for conditions like autism."

Gerard Clarke, a microbiome researcher at University College Cork in Ireland who was not involved in the study, tells *The Scientist* in an email that we can't definitively say whether "these very interesting associations manifest in biological or physiological terms of relevance to social behavior," but that the paper yields "a number of important clues as to who might be involved in the conversation between the gut and the brain."

—Amy Schleunes

PROFILE

# The Father of Autoimmunity

By revealing that animals could develop immune responses against their own tissues, Noel Rose established an entirely new scientific field.

BY DIANA KWON

Science is full of ideas that have been proven wrong. Up until the 1950s, one prevailing view among scientists was that the body could not produce antibodies against itself. This concept, known as *horror autotoxicus*, or the fear of self-toxicity, was coined in the 19th century by Paul Ehrlich, a German physician-scientist who was awarded a Nobel Prize for his contributions to immunology.

Nearly half a century later, *horror autotoxicus* was overturned by Ernest Witebsky, a protégé of one of Ehrlich's trainees, with the help of Witebsky's student, Noel Rose.

When Rose joined Witebsky's lab at the University at Buffalo, a State University of New York (SUNY) school, in 1951, Witebsky was studying organ-specific antigens—molecules that make different cell types functionally distinct. Witebsky was particularly interested in thyroglobulin, a large protein found exclusively in cells of the thyroid gland, and he gave Rose the task of identifying the properties that made this molecule unique to the endocrine organ. Rose extracted and purified the protein from various animals—including horses, pigs, and humans—and mixed each with Freund's adjuvant, a solution containing dead bacteria that helps stimulate an immune response, before injecting it into rabbits. In response, the rabbits generated antibodies against the thyroglobulin as their immune systems reacted to the foreign substance.

"It really struck me that all of these very different animals would generate an immune response in the rabbit," Rose says. Careful analysis of the thyroglobulin revealed that the protein was indistinguishable no matter which species it came from, raising a question for Rose: How was the rabbit able to distinguish its native thyroglobulin from the injected protein? He decided to repeat his experiment using thyroglobulin from a rabbit. Presuming the protein to be identical across all rabbits, Rose extracted it from one animal and injected it, along with Freund's adjuvant, into another. Lo and behold, the rabbit that received the injection produced antibodies against the thyroglobulin derived from the donor rabbit.

When Rose first showed the results to his advisor, Witebsky was in disbelief. "He said, 'This is crazy. No one will believe this. How could this be?'" Rose recalls. Witebsky suspected that there must have been a mistake—that the thyroglobulin must have been denatured, for example. The young scientist went back to the bench and repeated the experiment, this time, using a more cautious method to ensure the protein was preserved. The results were the same. Following that, Witebsky told him to repeat the experiment once more, this time extracting thyroglobulin from a rabbit and injecting that protein back into the same animal. Even then, the animals developed antibodies against the protein when it was taken from the thyroid and introduced to another part of the body. "That was enough for Witebsky," Rose says.

Some other scientists—including journal editors—refused to accept the findings at first. It took six years to publish the work, but when it finally appeared in print, the results shook the foundations of immunology. Rose had proved *horror autotoxicus* was wrong.

"In every aspect, he is the father of autoimmunity," says George Tsokos, a professor of rheumatology at Harvard Medical School. "The man opened a whole chapter in the book of medicine."

Rose is more cautious about saying his work completely overturned Ehrlich's on autoimmunity, explaining that Ehrlich's original quote on *horror autotoxicus* may actually have been misconstrued. "If you read the whole paragraph in which he proposed this idea, he really never said that you couldn't produce autoimmunity," Rose says. "He said that if you produce it, it may be dangerous. So in many ways, Ehrlich may have been right."

## DIVING INTO THE MICROSCOPIC WORLD

Rose grew up in Stamford, Connecticut, and caught his first glimpse into the hidden world of microorganisms in seventh grade. One of the science teachers at his school would bring in a personal microscope—and some of the students, including Rose, spent their spare time peering through it. The tiny creatures they saw captivated their young minds. "I became enraptured with the idea that there is another world around us that we don't see," Rose says. "It was something that raised my curiosity from the beginning and has been the theme of most of my career."

After high school, Rose was accepted into a bachelor's program at Yale University. It was the mid-1940s, and his father, a physician who had just returned home after serving as a medical officer during World War II, was reestablishing his clinic. This meant the family had little money to spare—but Rose was able to obtain a scholarship to help cover the cost of his education. "Without that, I certainly would not have been able to attend," Rose says. "Those were very hard days."

When Rose began his undergraduate studies, he wanted to pursue microbiology. But it was not yet a well-developed discipline, and there weren't many courses on the subject, so Rose majored in zoology and took the microbiology classes as electives. Those classes were taught by researchers in the botany

COURTESY OF NOEL ROSE AND JHU

## CAREER TITLES AND AWARDS

Emeritus Professor, Johns Hopkins University
Part-time Senior Lecturer on Pathology, Brigham and Women's Hospital
Golden Goose Award, American Association for the Advancement of Science (2019)
Founder and Director, Johns Hopkins Autoimmune Disease Research Center (1999–2015)
Chair, Autoimmune Diseases Coordinating Committee, National Institutes of Health (2003–05)
Nicolaus Copernicus Medal, Polish Academy of Sciences (2009)
Keystone Lifetime Achievement Award (2006)
Elected Fellow, American Association for the Advancement of Science (1999)

### Greatest Hits

- Discovered that rabbits could develop an immune response to their own thyroglobulin, the first demonstration of autoimmunity
- Revealed that the susceptibility to an autoimmune disease of the thyroid gland in mice is determined by genes encoding for the murine major histocompatibility complex
- Identified that a virus, coxsackie B, plays a role in causing inflammation in the heart in humans

department, because bacteria were largely regarded as members of the plant kingdom at the time. It was in that department Rose met his first mentor: a doctoral student named Joshua Lederberg who would later go on to win a Nobel Prize for his discoveries in bacterial genetics. In those days, Lederberg let Rose and his classmates spend time in the lab where he worked, learning about his research. "He taught us how to think as scientists," Rose says.

**I became enraptured with the idea that there is another world around us that we don't see. It was something that raised my curiosity from the beginning and has been the theme of most of my career.**

—Noel Rose, Brigham and Women's Hospital

After completing his undergraduate degree, Rose was torn between going to medical school or to graduate school to focus on basic research. Rose had developed a strong interest in medicine due to the influence of his father. But the microscopic world still fascinated him and tugged at his desire to do research. MD/PhD programs weren't an option back then, so Rose took the advice of a member of the medical school admissions committee at Yale, who suggested he obtain a PhD and then teach while working on a medical degree.

Rose started his PhD in microbiology at the University of Pennsylvania in 1948. At Penn, he joined the lab of the microbiologist Harry Morton and studied the bacterium *Treponema pallidum*, which causes syphilis. Culturing the organism and examining it under an electron microscope, Rose discovered that the microbe had flagella-like structures on its surface that drove it to whirl in a corkscrew motion.

## *HORROR AUTOXICUS* OVERTURNED

While foraying deep into the world of microbiology research, Rose took premed courses with the intention of attending medical school. After obtaining his PhD, he was accepted into medical school at the University at Buffalo (UB) and moved there with his wife, Deborah, to earn his MD while teaching classes on the side for a small salary and waived tuition fees.

Rose arrived at UB in 1951. At the time, Ernest Witebsky, an immunologist who had fled Germany in 1936 to escape the Nazis, was one of the star scientists on campus. Witebsky's claim to fame

was research from his days at the University of Heidelberg, where he had characterized the cellular features that distinguish each blood group and act as antigens if introduced into individuals of differing blood type. Rose was drawn to Witebsky's work and joined his lab, and it was during his first few years there that he conducted the critical experiments with thyroglobulin and rabbits, overturning the dogma of *horror autotoxicus*. But this was only the beginning of the story.

Rose went on to investigate the consequence of developing an immune response to substances made by one's own body. To do so, he removed and studied the thyroid glands of rabbits that had been injected with their own thyroglobulin. Analyzing the tissue showed that the body's own immune cells, recruited by antibodies, had infiltrated the organ and damaged it—and, in some cases, completely destroyed it. "That was really the major discovery," Rose says.

Even though Rose and Witebsky knew they had landed on something big, the first journal to which they submitted their results dismissed them as utterly impossible findings—and told the authors they were probably mistaken. So Rose, Witebsky, and their colleagues went back to the lab to do more experiments. This time, their work linked the animal data with a human illness—Hashimoto's disease, a rare condition marked by an inflamed thyroid gland (thyroiditis) that, at the time, had no identifiable cause. To make the connection, the team had examined serum samples from patients and tested them against human thyroglobulin and found that the blood developed the same type of antibodies identified in rabbits injected with their own thyroglobulin (*JAMA*, 164:1439–47, 1957).

"We went ahead and showed that this same destruction applies to humans and that you could induce a disease in an organ by immunizing it with a specific antigen of the same species," Rose says. "And that was autoimmunity."

## UNRAVELLING THE ORIGINS OF AUTOIMMUNITY

"The findings and the work of Dr. Rose in those early years really set the stage for our understanding of autoimmune disease in the human," says Joseph Bellanti, a professor emeritus in microbiology and immunology at Georgetown University. Bellanti, who was a medical student at UB when those discoveries were made, says that both Rose and Witebsky, and their work, influenced his own decision to pursue immunology research.

The work influenced many other researchers as well. In labs around the world, researchers began looking into other diseases where inflammation appeared with no apparent cause. In many cases, these turned out to be autoimmune diseases. The discovery also led to the swift rise of Rose's career. Shortly after publishing the autoimmunity results in *JAMA* in 1957, he was invited to spend several months in Europe giving lectures about the discovery. "Suddenly, work came out of the walls," Rose recalls.

Although he had proven himself a keen researcher, Rose did complete his MD in 1964 and remained at UB as a physician-scientist. During this time, he made another, somewhat serendipitous discovery. At a meeting at the Jackson Memorial Laboratory in Bar Harbor, Maine, he met researchers who were using histocompatibility antigens—tissue cell–surface "ID" molecules that, when closely matched, increase the success of organ transplants—to examine the genetic differences in cancer susceptibility in mice.

These antigens intrigued Rose because they seemed to offer a potential explanation for a puzzling finding: while most rabbits would develop an inflamed thyroid gland when immunized with thyroglobulin, some did not. Along with a postdoctoral fellow in his lab, Adrian Vladutiu (who passed away in 2014), Rose used mice to examine whether there was a gene that made the animals more vulnerable to the inflammation. Vladutiu's experiments revealed that differences within the genes encoding for the murine major histocompatibility complex, H-2, determined how susceptible the mice would be to thyroiditis (*Science*, 174:1137–39, 1971).

> **In every aspect, he is the father of autoimmunity. The man opened a whole chapter in the book of medicine.**
>
> —George Tsokos, Harvard Medical School

As his research progressed, Rose took a professorship at Wayne State University, where he remained for nearly a decade before joining the faculty at Johns Hopkins in 1981. At Hopkins, Rose shifted his focus from nature to nurture, searching for environmental, rather than genetic, triggers of autoimmune diseases. "There was still a big void—that is, even in the best-studied examples of thyroiditis, genetics was always less than half the risk," Rose says. "We thought something from the environment must be involved."

The group decided to focus on myocarditis, inflammation in the heart, because clinical evidence showed that patients typically had infections before developing the condition. Myocarditis has remained a primary focus of Rose's lab for decades, with his team's relentless work unravelling the causative roles of both genetics and a virus called coxsackie B.

For decades, Rose and his collaborators dug deeper into autoimmunity. In 2016, he became an emeritus professor at Hopkins. He and his wife moved to Boston, where he stayed connected to research, taking a part-time appointment as a senior lecturer at Brigham and Women's Hospital. Still searching for answers at 92, Rose is passionate about using big data to study autoimmune diseases. He sees great potential in examining large databases of patient data to get at the question of why people develop certain autoimmune diseases and what types of treatment would be best suited for interrupting the development of these conditions.

"What we want to do is avoid the train wreck from the beginning, and I think we can begin to do that," Rose says. "That's what I'm excited about." ■

# Janelle Ayres: Immunity Pioneer

Molecular and Systems Physiologist, Salk Institute for Biological Studies

BY AMY SCHLEUNES

Growing up in Livermore, California, Janelle Ayres kept all kinds of pets—rabbits, birds, fish, turtles, and her two favorites, Smokey the Siberian husky and Roman the German shepherd. She dreamed of becoming a veterinarian, but learning about genetics in high school led her to study molecular and cell biology at the University of California (UC), Berkeley. She then had to choose among vet school, med school, and a PhD program. Attending a talk by Stanley Falkow, whom she calls "the godfather of microbial pathogenesis," helped her decide to pursue microbiology. "I loved the idea of host-microbe interactions," Ayres tells *The Scientist*, "and that pathogens could be having such dramatic effects on the host's biology."

After graduating in 2002, Ayres moved to Stanford to work with microbiologist David Schneider. He encouraged her to follow her own ideas, and after many hours spent "PubMed wandering," she kept circling back to the same question: What does it mean to survive an infection? The dogma then was that surviving an infection required killing a pathogen, and while that's true in many cases, Ayres says, it didn't account for all infections.

Wheat, for example, can tolerate certain pathogens without treatment, leaving Ayres to wonder: if plants could endure infections, did animals also have this disease tolerance? To investigate, she injected fruit flies with *Listeria*. All of the flies, whether they died or not, had similar levels of bacteria, indicating that pathogen load or an inability to eradicate the infection wasn't what killed the insects. Ayres next analyzed the genetics of the flies and found tweaks in genes unrelated to the immune system that promoted tolerance of the infection. Animals, like plants, do tolerate disease, she showed (*Genetics*, 178:1807–15, 2008). (See "Taming the Beast," *The Scientist*, June 2019.) Working on that project, Ayres was "really engaged and thoughtful in a way that I now understand isn't very typical" of graduate students, Schneider says. She finished her PhD in 2009 and returned to UC Berkeley for a postdoc in the lab of microbiologist and immunologist Russell Vance. Four years later, she opened her own lab at the Salk Institute for Biological Studies in La Jolla, California.

There, Ayres set out to investigate whether disease tolerance existed in mammals. She worked with mice, which tend to lose muscle mass after infection with *Salmonella* or *Burkholderia thailandensis*. And, just as in her earlier fruit fly experiment, she found that some mice experienced muscle wasting while others of the same strain didn't, even when the animals had similar pathogen levels. It turned out that a particular strain of *E. coli* in the microbiomes of surviving mice had helped them tolerate infection. Giving that *E. coli* strain to infected mice as a probiotic protected them against muscle wasting (*Science*, 350:558–63, 2015).

"Janelle's work is remarkably creative in identifying mechanisms of tolerance and really digging deep and trying to understand how the mammalian host . . . can actually withstand typical bacterial infections," says Dan Littman, an immunologist at New York University who has tracked Ayres's work. "She has the ability to really think around problems without being held back by the conventional wisdom in the field."

In a 2017 *Cell* paper, Ayres and her colleagues showed that when *Salmonella typhimurium* infects a mouse, the bacterium uses an effector protein called SlrP to manipulate the animal's gut-brain axis. The mouse continues eating as a result, whereas with other infections the animal would lose its appetite. This manipulation of the animal's behavior promotes host health and survival, along with pathogen transmission, Ayres says (*Cell*, 168:503–16.e12, 2017).

In a more recent experiment, she and her team analyzed gene activation in genetically identical mice infected with *Citrobacter*. Mice that survived had increased expression of genes regulated by iron. When her team fed iron-enriched chow to a new group of infected mice, 100 percent of the animals survived, despite the pathogen's persistent presence in their organs (*Cell*, 175:146–58.e15, 2018).

"Every experiment seems to surprise us," Ayres says, "and we're committed to pushing this field forward." The goal is translating the work into humans. When a patient is infected with a particular pathogen, she explains, "I want to be able to define how I can intervene and shift them back onto a trajectory to a healthy state." ■

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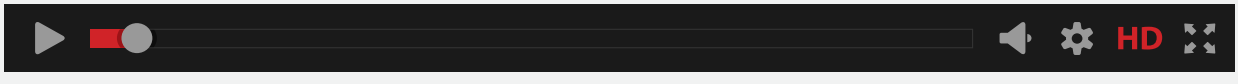


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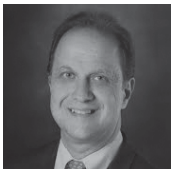

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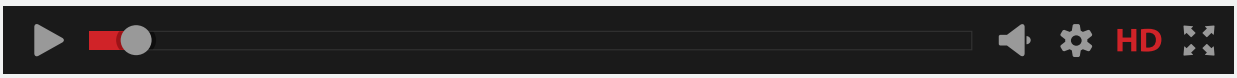

# The Ripple Effects of Misconduct

What happens to the students and early-career collaborators of a senior investigator accused of scientific malpractice?

BY KATARINA ZIMMER

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Evolutionary ecologist Kate Laskowski didn't have a good start to her new faculty position at the University of California, Davis. She was just a few months in when, late last year, she received an email from a researcher who had some concerns about a study she had coauthored in 2016 with the prolific McMaster University spider biologist Jonathan Pruitt.

As a graduate student at the University of Illinois, and later a postdoc at the Leibniz Institute of Freshwater Ecology and Inland Fisheries in Germany, Laskowski had collaborated with Pruitt on a study of spider social behavior. The email noted that the raw data collected in Pruitt's lab, then at the University of California, Santa Barbara, contained odd duplicate values in the columns of a spreadsheet that documented behavioral differences among individual spiders. After combing through the data herself, Laskowski ultimately came to the conclusion that the data, and the study based on them, weren't trustworthy, and requested earlier this year that the *American Naturalist* retract the paper. She'd go on to retract another two, both of which she'd coauthored with Pruitt.

These studies—which supported the hypothesis that the behaviors of individual spiders are influenced by social interactions—would be the first of several of Pruitt's papers to come under scrutiny from scientific journals, in a series of retractions and expressions of concern that has rattled the animal behavior research community and affected numerous collaborators, including many students and early-career researchers. "I'm in my first year of . . . my dream job," Laskowski tells *The Scientist*. "I've been so excited to set up new projects, and then I've had to spend the past four months dealing with all of these old papers that I thought I was over and done with."

McMaster University's investigation into Pruitt's publications is ongoing, and conclusions have yet to be drawn about whether the data oddities are accidental or due to manipulation. (Speaking to *Science* in January, Pruitt implied the issues were due to data mismanagement.) Yet the ensuing conversation within the scientific community has raised the question of what happens to early-career researchers when their senior collaborators or supervisors are accused, or worse, found guilty of research misconduct.

Through no fault of their own, many graduate students and postdocs end up as collateral damage in such situations, says Wanda Jones, associate director of research integrity at the US Office of Research Integrity (ORI), which oversees institutional investigations into scientific misconduct. Among the 30 or so investigations for which the ORI returns a finding of misconduct each year, around 40 percent conclude that principal investigators (PIs) were the ones responsible, while the rest point the finger at students, technicians, and other staff, Jones says. In a given year,

dozens of young researchers may find their research projects in jeopardy, or have to scramble to find new positions when their labs shut down—a challenge made particularly difficult by the retractions that often accompany misconduct allegations.

"There may be tremendous misfortune that plays out among the postdocs, among the grad students, among the staff of the principal investigator found to have committed research misconduct," Jones says.

## Trust shaken

The most immediate impact on early-career researchers affected by a misconduct scandal is often psychological, Jones notes—from worries about one's scientific reputation and career prospects, to a crisis of trust in collaboration and in science as a whole. "You could see how this could be traumatic for a student," says Russell Tracy, a biochemist at the University of Vermont Larner College of Medicine. "They may just decide that they want to do something else with their life and not do this anymore."

That's what Mary Ann Allen first thought in 2006 when she and five other graduate students discovered that their supervisor, biologist Elizabeth Goodwin at the University of Wisconsin–Madison, had falsified results on grant applications. The students ended up turning Goodwin in to the university administration, in an agonizing ordeal that they described to *Science* later that year. The university's subsequent verdict that Goodwin had committed fraud led Allen to conclude that data fabrication was likely a lot more common in the scientific community than she had presumed. "When that happened—and this is true for almost everybody I've talked to who's been in one of these situations—you come to the conclusion that the majority of science is falsified," she says.

Allen's reflexive reaction was to consider quitting grad school. But when she reached out to a researcher at another institution to ask for a reference to pursue a computer science degree, he gently encouraged her to continue in science, she recalls. "I've come back from that now because I've worked with a lot of labs who work hard to get their research," she says. "But at that moment . . . it [was] the idea that you don't trust science anymore." As for Allen's fellow students, three of them left graduate school before completing their PhDs, although *The Scientist* was unable to reach them to ascertain if that was because of the experience with Goodwin.

**You could see how this could be traumatic for a student.**

—Russell Tracy, University of Vermont Larner College of Medicine

## Moving labs

Allegations of misconduct against a PI raise complex logistical challenges in addition to existential ones for the people working in his or her lab. If a PI's position is terminated, students have to find new scientific homes—although often, universities try to relocate students to new laboratories within the institution, notes Alexander Runko, the director of ORI's division of investigative oversight.

In the case of Goodwin's lab, the thesis committees of the two students aside from Allen who decided to continue graduate school helped find new labs for them, Allen recalls. One of the students had to adopt a new research project, while the second insisted on continuing the research she had pursued under Goodwin's supervision. Allen herself found a position in a new lab through a postdoc she knew. She ended up switching from studying genetic sex determination in *Caenorhabditis elegans* to researching RNA modulation, "a field I had been interested in anyway," she recalls. In all three cases, the transition added years to the students' PhD programs, although all eventually graduated.

Staying in science can get particularly complicated when student visas, scholarships, or funding are tied to a particular investigator, Jones notes—although again, universities usually try to help where they can. Allen's and her fellow grad students' funding had been in Goodwin's name, and when Goodwin was found guilty, the University of Wisconsin returned the sum to the federal government. Ultimately, the School of Medicine and Public Health came up with some funds to support the group, Allen recalls. The University of Ulm in Germany did something similar for graduate students who had been working in the lab of Friedhelm Herrmann and Marion Brach, two cancer researchers accused of data manipulation in 2000 (although both denied guilt). Eberhard Hildt, who raised the alarm about the scandal when he was a postdoc in their lab, tells *The Scientist* that the university stepped in to replace the lost financial support.

The practical problems don't end when students or postdocs are ready to leave the institution. Applying to positions elsewhere can be fraught for those coming from a lab whose reputation has been tainted by fraud allegations, even when they aren't embroiled in the scandal themselves, Hildt notes. "It's kind of a catastrophe because they are coming from a lab that is famous for scientific misconduct, and this is not the best reference," he says. In 1997, when Hildt was growing suspicious over Hermann's and Brach's research practices, he confided in a former supervisor who had overseen his PhD thesis. That person not only supported Hildt in blowing the whistle, but also provided references for new job applications, says Hildt, now an investigator at the Paul Ehrlich Institute near Frankfurt.

## Retraction troubles

Neither Hildt nor Allen had published research with their supervisors. But others have found themselves in situations where data manipulated or fabricated by senior collaborators have made their way into joint publications that end up getting retracted, creating unique challenges for young researchers whose careers are just getting off the ground. "It's terrify-

ing in some cases when stuff that you've contributed to, that you believe in, is now being retracted," says Katharine White, a chemical biologist at the University of Notre Dame Harper Cancer Research Institute in Indiana. White witnessed a case of misconduct as a grad student at MIT, where a senior postdoc had falsified data—although White wasn't involved in any of the postdoc's papers. "PIs [often] manage to survive it. I don't know if a lot of graduate students who are caught up in it survive it."

Lost publications were a major concern for junior members of the lab of Eric Poehlman, an obesity and aging researcher at the University of Vermont who in 2005 pleaded guilty to charges of data fabrication in studies and grant applications. After losing several manuscripts to retractions, Poehlman's postdocs suddenly had little to show for more than a year's worth of research.

Worried about how the loss would affect future applications for new positions and grants, the University of Vermont's Tracy, then the senior associate dean for research and academic affairs at the school, got together with other senior faculty to advise the postdocs on how to explain the situation in their CVs and in grant proposals. He also wrote letters to grant officials at the National Institutes of Health "to let them know that it's unfortunate, but a lot of their hard work isn't being represented in their bio sketches because of the malfeasance of Dr. Poehlman," he recalls. "What impact those letters had I don't know, but we felt as an institution it was part of our job to help our young investigators build their careers."

Retractions related to misconduct allegations can affect researchers years after the papers themselves are published. In 2009, a European researcher, who asked to remain anonymous because of the sensitivity of the issue, was a few years into a job at a biotech firm when a paper they had coauthored as a PhD student with the Danish neuroscientist Milena Penkowa was retracted. On top of the "painful" experience of having to comb through raw data collected seven years earlier in order to assist an investigation into Penkowa's research practices—and to clear their own name—the anonymous researcher says they worried that the retraction would prompt the University of Copenhagen to invalidate

> **I was of course afraid that [it] could end up with me being fired from my job.**
>
> —An anonymous researcher whose supervisor was accused of misconduct

their PhD thesis because the study had formed a key part of it. The researcher also feared that the widely publicized investigation—which resulted in a "blatant forgery" charge for Penkowa (later overturned on appeal)—would influence their employers. "I was of course afraid that [it] could end up with me being fired from my job because they then wouldn't trust in me," the researcher recalls.

Fortunately, their former PhD supervisor took action to ensure that their thesis wasn't affected by the process, and the researcher was able to reassure their employers that they hadn't played a role in the suspect part of the retracted paper. Nevertheless, when they apply to new positions, they only display their "top five" publications, the researcher says, because they don't want potential employers to immediately associate them with Milena Penkowa.

## Positive change

Despite the obstacles they create for everyone involved, misconduct investigations and the conversations they inspire can spur positive change, not least at the institutions that handle such situations. For instance, in the wake of the investigations into Goodwin's actions, the University of Wisconsin–Madison introduced an official policy to help relocate students to new laboratories and secure funding should similar situations with other PIs arise in the future. When Allen started a faculty position at the University of Colorado Boulder, she helped the institution set up a similar policy.

For the young researchers themselves, coming face-to-face with alleged or proven scientific misconduct can be transformative, making them more-diligent scientists and educators. Laskowski, for instance, now gives any data collected as part of her research a full "strip search" during analysis, though she will continue to trust her collaborators, she says. And Hildt tries to encourage his students to be open-minded about the outcome of experiments and to avoid mindsets that could lead to data fabrication. White and Allen say they've instated similar practices.

Even people who aren't involved in the affected labs can learn from watching incidents unfold from afar. Alexandra McInturf, a PhD candidate studying animal behavior at the University of California, Davis, says the ongoing Pruitt investigations have made her reflect on the publish-or-perish mindset of academia—a thought process she documented in a widely praised blog article back in February. "I hope this Pruitt data debacle, for whatever reason it was caused, sparks a lot of really good momentum in the future to create better science, even if it's at a slower pace," she tells *The Scientist*.

Allen, who now teaches a class on responsible research conduct alongside her courses on RNA's roles in disease at Boulder, says that this desire to create a better community is common among people affected by the issues surrounding scientific misconduct. "If you stay in science, which not everybody does, you champion good science." ■

*Katarina Zimmer is a New York–based freelance journalist. Find her on Twitter @katarinazimmer.*

### WHAT TO DO IF YOUR SUPERVISOR IS SUSPECTED OF RESEARCH MISCONDUCT

**If the suspicions are your own, let someone know:** The Office for Research Integrity (ORI) advises people to contact the ORI, institutional research integrity officers, journal editors where publications are concerned, and funding agencies for grant applications. Often, anonymous complaints are possible "if [whistleblowers] have a fear of retaliation," says the ORI's Alexander Runko.

**Shore up professional connections:** Find other senior researchers at your institution whom you trust—thesis advisory committee members or department chairs, for example—who can vouch for you, write references, and offer guidance, says Mary Ann Allen, an RNA biologist at the University of Colorado Boulder. "I know networking is hard," she says, "but that really helps more than anything in these situations."

**Read your institution's policies:** Many institutions have policies to protect whistleblowers, and sometimes to help students find new positions. "Make sure that you understand what your institution is supposed to be doing for you," says Russell Tracy, a biochemist at the University of Vermont.

**Be transparent:** If you've lost papers to retractions, or have publication gaps, you can explain why and highlight your role in retracted papers in your CV or on funding applications, notes Katharine White, a cancer biologist at the University of Notre Dame. "People respond to people that can talk knowledgeably and confidently about their results."

**Talk about it:** Kate Laskowski of the University of California, Davis, recommends speaking with a licensed counselor, friends, or other affected students. "Seek out as much support as you can from other people."

# Revolutionary Repurposing

Evolution needn't make improbable leaps to facilitate transitions into uncharted biological territory. Adapting new uses for existing structures works just fine.

BY NEIL SHUBIN

You might think that lungs arose when ancient fish evolved to live on land and that feathers came about as the reptilian ancestors of birds took flight. You would not be alone. But you would be entirely wrong. These remarkable adaptations evolved long before the functions for which they are now well known. And they are not exceptions; they illustrate general principles behind many of life's great revolutions, ones that apply to the origins of organs, tissues, and even DNA.

I explore these principles and how they've functioned over billions of years of evolution in my latest book, *Some Assembly Required*.

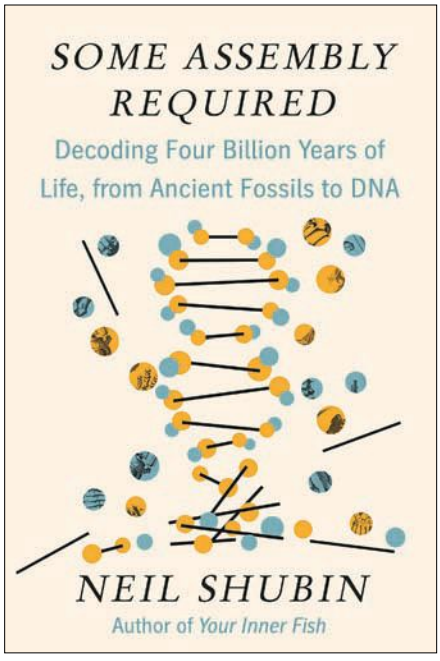
Consider vertebrates' transition from life in water to life on land, a shift that happened more than 370 million years ago. For the descendants of fish to adapt to their new terrestrial lifestyle, virtually every anatomical feature had to change. Life on land requires limbs with numerous joints, a breathing apparatus that doesn't accumulate carbon dioxide in air as gills do, and feeding structures that do not depend on generating a huge vacuum, something that is effective underwater but nearly useless elsewhere.

Because the list of features that are involved in this evolutionary shift seems prohibitively long, at first glance such a transition from life in water to life on land would appear impossible. The same is true for other great transitions. Flight, for example, requires hollow bones, feathers, wings, and more. If creatures had to evolve numerous traits simultaneously to make these great revolutions, it seems likely that evolution would have stopped dead in its tracks several times throughout life's history.

Indeed, this very point was one of the most powerful critiques of Charles Darwin's original evolutionary models after his publication of *On the Origin of Species* in 1859. In response, Darwin proposed that the great revolutions in the history of life don't involve the development of completely new features, but rather the repurposing of existing features for new uses. Lungs, as he knew, first arose in fish living in water. Many species of fish have lungs that they use to breathe air when the oxygen content of water drops below a critical threshold. Moreover, most fish species have an air sac that lies adjacent to the esophagus. In some, the sac helps keep the animal neutrally buoyant; in others, it is vascularized and serves as a lung for gas exchange. We now know that lungs preexisted vertebrates' invasion of land by millions of years.

The fossil record also contains fish species such as *Tiktaalik roseae*, the fishapod, and other extinct fishes dating to more than 360 million years ago that have arm bones with shoulders, elbows, wrists, and stubs of bone that approximate fingers. *Tiktaalik* even had a neck, something that the majority of fish species, extant or extinct, lack. All of these features were present in creatures that were primarily aquatic, enabling them to move about, breathe, and feed in water. Fish didn't have to evolve new features to invade land. They just had to use existing features in new ways.

This repurposing, co-opting, duplicating, and modifying of ancient features for new uses seems to apply as much to genes and entire gene networks as it does to anatomical features and fossils. Genomic, computational, and imaging technologies have given us a new and powerful lens with which to view evolution. The more we look, the more we find that the origin of new organs comes about by co-opting genes and developmental processes that originally served another purpose in the body. Genes that are involved in the development of the body axis were redeployed during the origin of appendages and limbs, for example. And the gene networks at work during the formation of the dorsal fins of fish were later used to make fins elsewhere on the body.




*Pantheon, March 2020*

Evolution is like a lazy baker who modifies versions of the same recipe to make a diverse selection of pastries and breads. Fossils, genes, and embryos reveal the deep and surprising connections among all living things and show how simple shifts can launch entire revolutions that change the world. ■

*Neil Shubin is the Robert R. Bensley Professor of Organismal Biology and Anatomy at the University of Chicago.* *Read an excerpt from* Some Assembly Required *at the-scientist.com.*

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# Confronting a Pandemic, 1957

BY CATHERINE OFFORD

The first cases were reported in Guizhou province in southwestern China in 1956. By February 1957, the disease, a form of influenza that caused typical flu symptoms such as a sore throat and fever, had arrived in Singapore. Weeks later, it hit Hong Kong.

Maurice Hilleman, a microbiologist at the Walter Reed Army Institute of Research in Maryland, read about the outbreaks in *The New York Times* on April 17 of that year. An article entitled "Hong Kong Battling Influenza Epidemic" stated that 250,000 people there were receiving treatment for the infection. Lines of people, including "many women" carrying "glassy-eyed children," were forming outside health clinics, the article noted. "I said, 'My God, this is the pandemic. It's here,' " Hilleman recalled in an interview decades later.

On April 18, Hilleman cabled a US military lab in Japan and managed to procure saliva from a patient infected in Hong Kong. His team quickly isolated the virus and tested it against hundreds of samples in Walter Reed's blood bank. The results confirmed Hilleman's fears: none of the samples neutralized the virus, a sign that none contained antibodies against it. This appeared to be a new influenza strain. Left unchecked, Hilleman predicted, it would reach the US within months, with disastrous consequences.

Hilleman, who had previously worked on vaccines for other influenza strains, convinced pharmaceutical companies to start on a vaccine right away, bypassing the US Division of Biologics Standards, the agency regulating vaccine development at the time. A Montanan who'd grown up on a farm, Hilleman also persuaded chicken farmers not to kill their roosters, as they usually did each spring—a move that ensured researchers had enough fertilized eggs to incubate the pathogen, then a standard step in vaccine development.

AROUND THE WORLD: The H2N2 strain of influenza caused a pandemic that killed at least 1 million people worldwide between 1956 and 1958. Here, more than 150 men in the Swedish military sickened with the disease rest in a converted gym in Luleå in northern Sweden.

When the virus, later named H2N2, reached the US that summer, the country was ready. By the fall, several million people had received the vaccine and tens of millions more doses were distributed. Hilleman's vaccine likely saved hundreds of thousands of lives before the pandemic burned out in 1958, says Paul Offit, a pediatrician and vaccine specialist at the University of Pennsylvania's Perelman School of Medicine who wrote about Hilleman in his 2007 book, *Vaccinated*. All told, around 100,000 people in the US and more than 1 million worldwide died from the disease, popularly known as the "Asian flu."

Hilleman went on to develop many more vaccines, including 9 of the 14 now routinely administered to US children. Writing in 2007, two years after Hilleman's death, National Institute of Allergy and Infectious Diseases Director Anthony Fauci described him as "perhaps the single most influential public health figure of the twentieth century."

Researchers battling COVID-19 face a greater challenge than Hilleman did with H2N2. There are no vaccines for any coronavirus, let alone SARS-CoV-2. But Offit suspects that, were Hilleman alive today, "he would have exactly the right idea for how to make the vaccine and how to make it quickly. . . . He would cut right through it—much as he did in 1957." ■

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